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COMPARATIVE EFFECTS ON THE CIRCULATORY SYSTEM OF POSITIVE AND NEGATIVE ACCELERATIONS THE MAREY "LAW"¹

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IN EARLIER reports on the effects of high acceleratory forces many disturbances of bodily mechanisms have been considered (1-4). Further inquiry has now been made into the circulatory changes induced by negative as well as positive accelerations. Arterial pressure levels in both carotid and femoral vessels have been followed simultaneously, and correlated venous pressure and cardiac changes have been studied. Results of tests on different animals indicate that under conditions of circulatory stress a distinct functional advantage is possessed by the primate form.

METHODS

Descriptions are given in previous reports of the apparatus and methods employed. The acceleratory forces used generally ranged from 2 to 6 g, developed by a centrifuge with 10-ft. rotor arms carrying animal subjects and electrical and other leads. A mercury manometer system which recorded electrically was utilized, and evaluation of the results was made by calibration with a U-tube manometer. In all cases measurements were made by the open vessel method, and non-experimental disturbances in circulation were thereby minimized. Blood flow was examined by a flow-meter based on the Venturi principle, previously described. Manometric systems with aqueous solutions were employed in the arterial systolic and diastolic and venous pressure studies.

The experiments were performed chiefly on monkeys (*M. rhesus*) and dogs. Local or light general anesthesia (ether or sometimes amytal) was employed for carrying out the superficial incisions to reach and cannulate the vessels; time was then

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allowed for recovery of the animal before the main studies were made. Heparin was given occasionally during tests. No exposures were pushed to lethal limits; in most

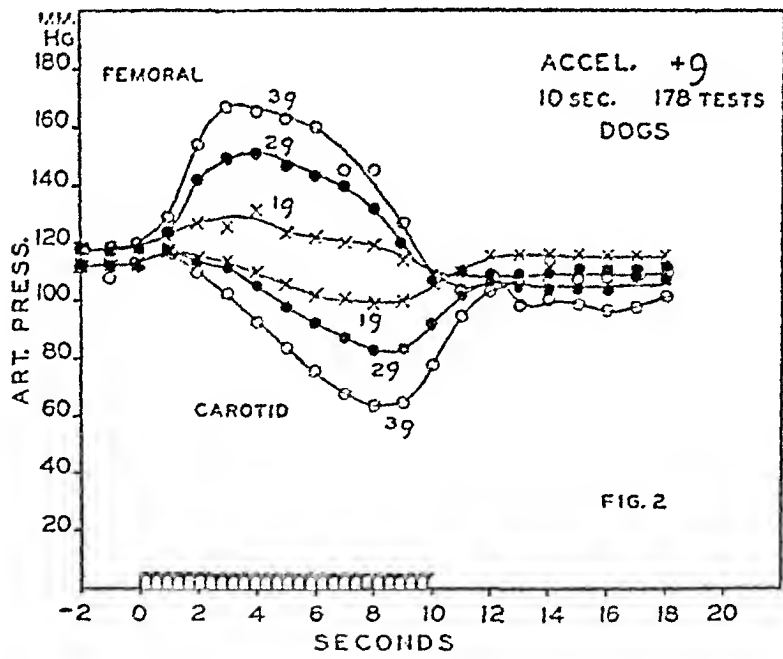
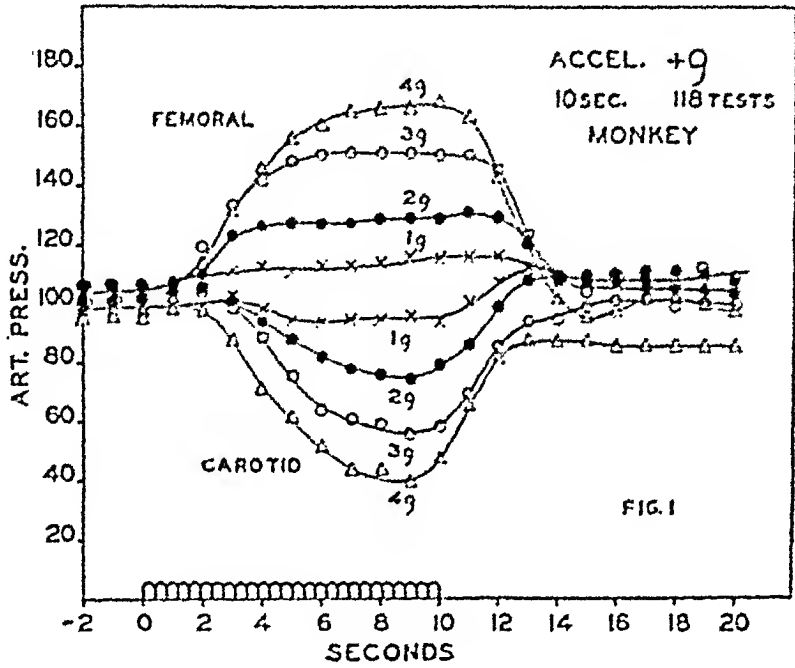
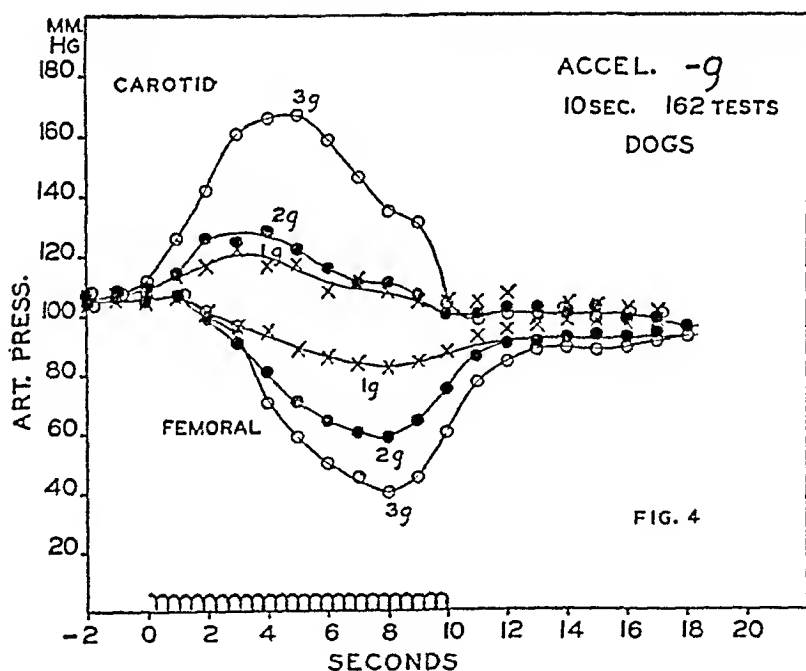
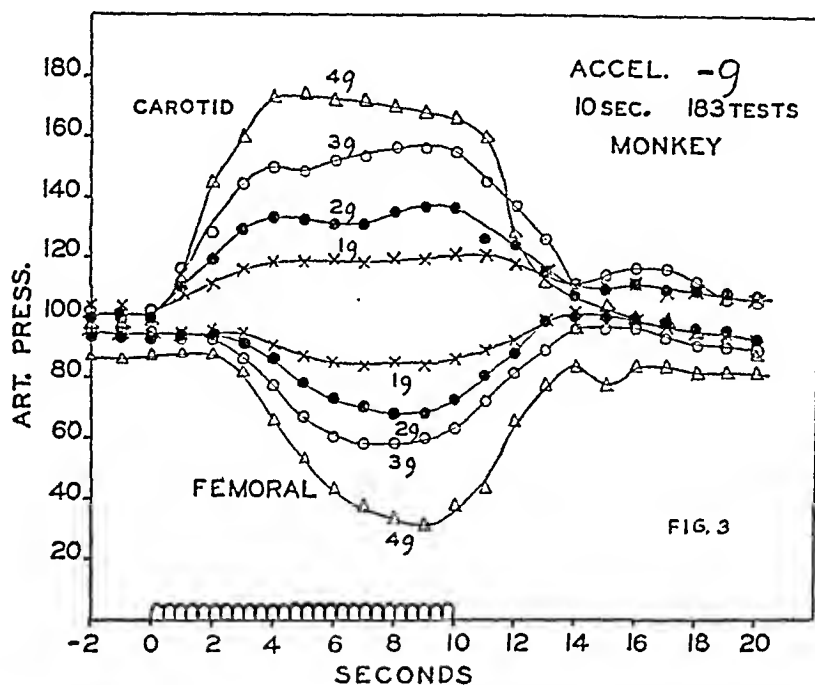


Fig. 1, 2, 3, 4. CAROTID AND FEMORAL ARTERIAL BLOOD PRESSURE CHANGES in the monkey and dog under positive and negative acceleratory forces. Graphs represent averages of all runs plotted from second to second. Usually from 10 to 25 runs were made at each g level.

cases they were light or moderate in degree, and after the first few trials animals were usually quiet throughout. Typical records derived from tests on the centrifuge have been published (4).

RESULTS

Arterial Pressure. Exposure to positive g produces a fall in carotid and a rise in femoral arterial pressure; when negative accelerations are imposed on the animal, the changes are opposite in direction. The relative magnitudes, time factors and



other characteristics of these changes have been studied in experiments made at different g levels. In figures 1 to 4 are given the results of over 600 tests on monkeys and dogs.

The changes in arterial pressure, traced from second to second and averaged,

show a fairly smooth reciprocity in the case of the monkey in both positive and negative g series. In comparison, curves of carotid and femoral pressures derived from the dog are not so consistent, and marked skewing is observed in all the tests. Maximal arterial pressure changes found at different g levels under positive and negative accelerations are shown in figures 5 and 6. The straight-line relationship is especially striking in the monkey. Results in the case of the dog again indicate less uniformity, however, the averaged points being not wholly in alignment. For a given g force, greater arterial pressure reductions are also observed in the dog.

It was noted in all animals after a few exposures to light g forces, and especially after a more severe test, that the arterial pressure commonly remained at subnormal levels for one to several minutes. These disturbances were seen in carotid, brachial and femoral values after both positive and negative g exposures. Arterial flow levels were similarly affected. Tachycardia was an accompaniment of these post-acceleratory changes. Some of these effects were noted by Poppen in 1932 (5), but remained unpublished in confidential files.

Systolic and Diastolic Levels. In a small group of experiments on dogs, using a water manometer, it was observed that the normal systolic-diastolic carotid pressure difference was equivalent to 20 to 30 mm. Hg. This difference was increased by low applied forces up to 2 g , and decreased on exposure to 3 g or higher levels. The overall effect of accelerations was of course a reduction in the mean systolic and diastolic carotid pressure values. After stopping the centrifuge there appeared within a few seconds, and for a brief period only, marked increase—up to 100 per cent—in the systolic-diastolic pressure difference. Carotid arterial blood flow variations under positive g stresses were roughly comparable to those of arterial pressure.

Minor Arterial Pressure Waves or 'Notches'. In our investigations over the past few years it has been observed that the carotid (or brachial) arterial pressure curve may show a small rise during the application of positive g forces. This rise appeared as a shallow wave or 'notch' of 5 to 10 mm. Hg at about 5 seconds after the start of centrifugation in the case of the monkey, and at 6 or 7 seconds in tests on the dog. Nearly 1000 records were examined (table 1).

The mean (overall) carotid pressure curve showed at this time marked depression; because of this fact, probably, the minor waves were frequently not apparent (obscured?) under the acceleratory forces used. The waves are also not well recognized in all the major curves shown herewith (fig. 1, 2) because of the smoothing effect of the main mass of data. The small waves occurred more frequently under lower than higher imposed forces; at 1 to 3 g they appeared in about one-third of the experiments, and at 4 to 6 g in about one-sixth of all tests. Their incidence was greater in the case of the monkey than in the dog under the higher accelerations. Reference is made later to their initiation probably through vasomotor or carotid sinus action.

The small arterial pressure waves or notches described should be distinguished from arterial pressure changes which accompany movements or struggle on the part of the animal. Those referable to the latter factors were larger and much more rapid fluctuations than the above; struggle effects were moreover clearly apparent in correlation with E.E.G., respiratory and other disturbances, and are not considered herein.

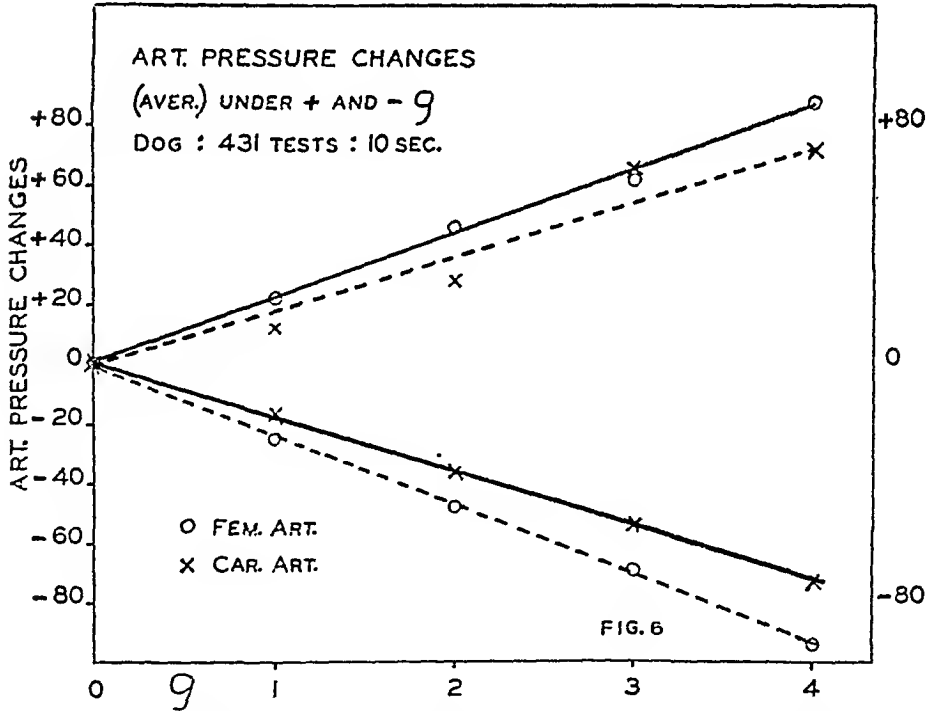
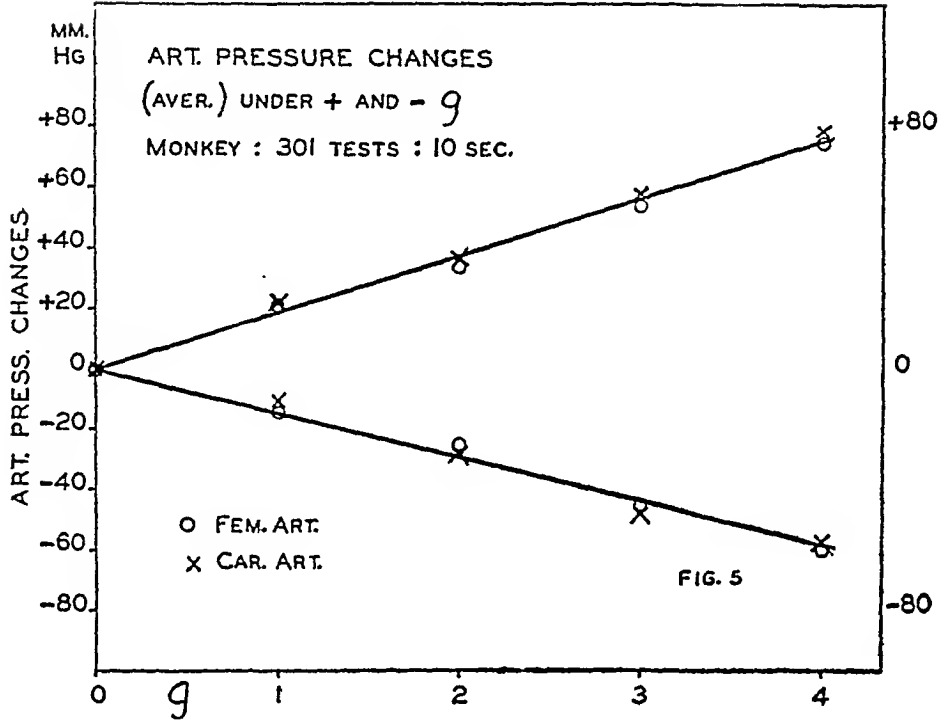


Fig. 5 and 6. ARTERIAL BLOOD PRESSURE CHANGES under positive and negative g forces. Maximal changes which occurred during all runs, at no specific time, are averaged, and results given in fig. 1-4 are included. The levels indicated vary slightly, therefore, from those shown in fig. 1-4. Each point represents from 10 to 30 runs. Total no. of tests—monkey 301, dog 431.

Arterial Pressure and Heart Rate Relationships. It was noted early in our experiments that a reciprocal relationship obtained between heart rate and arterial pressure under acceleratory forces. In positive g tests when carotid pressure fell the heart rate rose, and in negative accelerations the reverse relation held true. These rela-

tionships were specific and valid, it should be emphasized, in the case of tests carried out on the monkey only. The time-relations showed that heart rate increases followed carotid pressure falls by about 3 seconds in positive g tests, while under negative g forces the rate-pressure changes were opposing and almost simultaneous (table 2).

The reciprocal pressure-rate relationship did not hold in experiments on the dog and cat, except in the case of positive accelerations in the averaged results. In this $+g$ series the data nevertheless varied considerably, while under $-g$ they were markedly divergent. In a small group of experiments on the dog under amytal, also

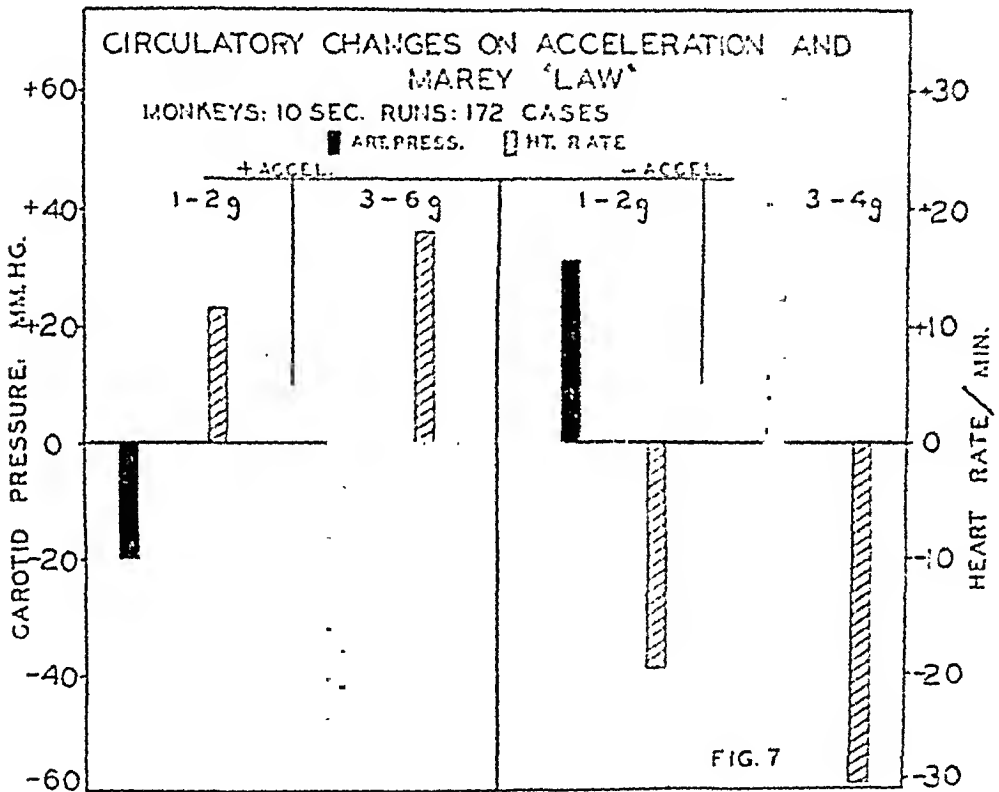


Fig. 7

no consistent arterial-pressure-heart-rate relations were found when pressures in the carotid or the femoral artery were suddenly raised.

Compared to femoral arterial pressure changes, pulse fluctuations were in the same direction in the monkey. The heart showed too, on the average, greater rate changes at the higher g forces; these changes were not so uniformly progressive as those found in earlier work, however, in which cardiac tests were made under somewhat different conditions (4).

For the same g forces used, heart rate and arterial pressure changes were significantly greater under negative than positive accelerations. In the case of negative g , arterial pressure fluctuations were also much more irregular throughout. Heart rate changes occurred much earlier, moreover, under negative g forces (tables 2, 3; fig. 3, 4).

Venous pressure. In a short series of tests on dogs it was found that under forces of 1 to 4 g for 10 seconds the pressure levels in the vena cava increased 50 to 100 per

TABLE 1. OCCURRENCE OF SMALL POSITIVE WAVES OR 'NOTCHES' IN ARTERIAL PRESSURE DURING ACCELERATION

ACCELERATION	DOG: NO. OF NOTCHES			MONKEY: NO. OF NOTCHES		
	Present	Absent	% Present	Present	Absent	% Present
1, 2, 3	60	137	30.5	88	201	30.5
4, 5, 6	20	151	11.5	49	256	16.0

Elevations of 5-10 mm. Hg, carotid and brachial arteries. All runs 10 sec. or longer. Total cases, dogs 368, monkeys 594.

In most instances, change (notch) in arterial pressure curve started in 5-6 sec. in case of monkey, and in 6-7 sec. in the dog.

TABLE 2. TIME AND EXTENT OF CIRCULATORY CHANGES DURING ACCELERATION

ACCELERATION	NO. OF TESTS	INITIAL CHANGE		MAXIMAL CHANGE REACHED			
		Heart Rate	Carotid Pressure	Heart Rate		Carotid Pressure	
g		sec.	sec.	sec.	beats/min.	sec.	mm. Hg
+1	23	4.2	1.4	8.0	+12	5.6	-11
+2	29	4.6	1.3	8.4	+11	6.7	-27
+3	25	3.4	1.3	8.9	+20	7.0	-44
+4	20	5.1	1.0	8.4	+15	7.2	-53
+6	10	5.0	1.5	7.3	+20	7.0	-61
-1	15	2.5	1.6	6.8	-21	8.0	+27
-2	16	2.9	1.8	6.3	-18	7.3	+35
-3	18	1.9	2.2	5.5	-32	7.3	+57
-4	16	1.9	2.1	6.2	-27	4.3	+67

9 Monkeys: 10 sec. runs.

TABLE 3. COMPARISON OF CIRCULATORY REACTIONS OF THE MONKEY AND DOG TO ACCELERATION

ANIMAL	ACCELERATION	NO CASES	CAROTID PRESSURE CHANGE	HEART RATE CHANGE
	g		mm. Hg	beats/min.
Monkey.....	Positive	45	-48	+18
Monkey.....	Negative	34	+62	-30
Dog	Positive	62	-63	+15
Dog	Negative	37	+69	+7

Exposure time, 10 sec.; 3g and 4g tests combined. No. tests: dog 99, monkey 79.

cent. Under the same conditions, pressures in the jugular vein fell only 25 to 50 per cent below normal.

Species differences in resistance to acceleration. For the two sub-primate animals

studied (dog and cat), carotid pressure decreases observed in 20 10-second tests at 4g averaged 80 mm. Hg. In 24 similar tests on monkeys, however, there was an average fall of only 58 mm. Hg.

A functional advantage was usually shown by the primate in both carotid and femoral arterial pressure values, under positive and negative accelerations; it varied from an equivalent of 0.5 to 1 g in the different tests employed (see also table 3 and fig. 4, 5). In the monkey, too, the reciprocal heart-rate-carotid-pressure changes, as well as the apparently more specialized reactions (carotid sinus, etc.), definitely set this animal apart in a higher physiological class.

DISCUSSION

The circulatory displacements which occur on exposure to acceleratory forces present to the animal a difficult functional problem. Because of the directly-opposed arterial pressure changes set up, the organism is faced with the need or opportunity of favoring one-half of its body against desirable and optimal conditions in the other half. The results above indicate that a specific type of response nevertheless comes into play, especially in certain mammalian forms. A reciprocal relationship between heart rate and carotid (or brachial) arterial blood pressure is clearly evident: under positive accelerations when the carotid pressure is reduced the heart rate is increased, and the opposite changes occur when negative g forces are used. With the concurrent femoral arterial pressure changes, it follows, the pulse rate is directly related.

In the primate only, it is found significantly, do these relations hold strictly in all cases. This animal, that is to say, shows rather remarkable reflex efforts to protect in the first place its cerebral circulation. In dogs (and cats) the results were widely variable in both the individual and the group, although the average levels showed in one respect—that under positive g stresses—opposing heart rate and carotid pressure changes. Evidently these lower forms are not so well-equipped reflexly as primates to combat the influence of high acceleratory forces. It is pertinent to recall here that all exposures were made with the animal in the fullp-out-stretched supine position.

The so-called Marey 'law' (6) considers that the heart rate varies inversely as the arterial blood pressure. This conception has been held valid under many conditions of life involving general arterial pressure changes in the whole body, although a number of exceptions have been noted in the literature. Its failure to hold in other cases, too, has been pointed out in a recent report (4).

The present work indicates that the Marey law should be strictly limited in the extent of its application. It appears indeed to call for a restatement, which may be put as follows: The heart rate is inversely and specifically related to the carotid arterial blood pressure (or carotid sinus pressure) in the primate animal. This expression, covering homeostatic responses usually found in the normal and otherwise resting animal, would appear to be in keeping with the lines of evidence now adduced. There is involved in the whole response, of course, a series of reactions through vagal, sino-aortic and other neural elements. In this connection it is worthy of emphasis that unanesthetized animals were used in the present experiments, and all neural and hormonal influences were kept intact. Koch (7) also has referred arterial pressure changes to carotid sinus mediation.

The reduction in carotid arterial pressure, it may be noted, occurred considerably earlier than the increase in heart rate in cases of positive g tests. Applied force in the opposite direction (negative g), however, brought about nearly simultaneous rate and pressure changes. Bradycardia under $-g$, that is, occurred much earlier than tachycardia under $+g$. Coupled with the greater (and irregular) changes in heart rate and arterial pressure under negative g , these findings point markedly to the more serious functional importance of negative rather than to positive accelerations of equal force. The lower tolerance to negative g and other facts mentioned heretofore give further emphasis to this hazard.

Differences which are observed in the monkey between carotid and femoral arterial pressures indicate that at the same g force an artery is more easily filled with blood than emptied (fig. 5 and 6). Whether the animal were placed in the head in or head out position (positive or negative g), that is, the distally-placed vessel from the centrifuge axis usually showed an earlier and greater blood pressure change. The difference could be accounted for only in very small part by the slightly greater g force applied to the more peripheral part of the animal. Calculations of g forces were made, it might be mentioned, by using the heart distance from the centrifuge axis as previously described. In the dog the results again were not always so clear—possibly explicable on the basis of the less linearly disposed body of the animal.

The small wave or notch in the arterial pressure curve which appeared from 5 to 7 seconds after the start of centrifugation suggested immediately reflexogenic activity. Almost certainly a carotid sinus reflex may be considered to be involved. The non-discernibility of this wave in a considerable percentage of cases investigated may be referable to the rigorous experimental conditions set up. The fact that the circulatory system nevertheless does respond against the severe acceleratory forces imposed implies an amazing potency on the part of the animal organism.

It seems fair to assume that in the case of man exposed to positive or negative acceleratory forces for 5 seconds or more, autonomic reflex action would be set in operation tending to offset the imposed forces. Such protective reflexes would have significant amelioratory value only, however, if the forces used were 2 g or less.

A comparison of results obtained from monkey and dog indicates that a distinct physiological advantage is possessed by the former animal. In the case of the monkey, *a*) there are smaller arterial pressure displacements under the same g forces, *b*) the reciprocity of heart rate and carotid arterial pressure changes is uniform and sensitively controlled, and *c*) carotid sinus reactions appear earlier and more often. Certainly the functional expressions of the primate form under circulatory stress are of a higher order than those exhibited by lower mammals.

The horizontal position in contrast to verticality allows a simpler body economy, especially on the basis of reflexogenic demands. Assumption of the upright position, although it gave so many and promises greater freedoms, imposed in the first instance a severe gravitational factor on the organism. Against it primates have fought a physiological battle over a long time-span. Even yet circulatory and other incompetencies—from headaches to ptoses to *pes planus*—are distressingly common in man. The infant's stumbling climb to uprightness foreshadows its inherent weaknesses, and it is clear that the curve of functional accommodation against gravity does not approach an asymptotic level even at adulthood.

Explanation of a present world dilemma may be made on the basis of man's relative poverty of cortical compared to rich mid-brain reactions. The baser among the latter, driving to wrangling and war, have so often reduced peoples to beggary; increasing reference of life's affairs to and their dominance by higher cortical levels should favor emergence, however, from our present profligacy. Knowledge that primates have progressed a significant functional (i.e. circulatory) distance along the way to uprightness leads at least to this cherished hope.

SUMMARY

The relative magnitudes, time factors and forms of carotid, brachial and femoral arterial blood pressure changes which occur under positive and negative accelerations up to 6 g are compared in the monkey and dog.

The simultaneous reciprocal reactions do not appear wholly smooth in the derived figures, but are nearly so in positive g tests, and better in monkey than dog. Curves secured from the latter animal showed a marked skewing, the pressure decreases in all series occurring later than the increases.

Arterial pressure and flow levels were commonly subnormal for one to several minutes after one severe or several light exposures. Fluctuations in systolic-diastolic difference in carotid arterial pressure and flow up to 100 per cent beyond normal occurred during the tests. Light exposures decreased and severe exposures increased the systolic-diastolic difference, while post-acceleratory values were markedly augmented.

Small positive carotid arterial pressure waves or notches of 5 to 10 mm. Hg occur 5 to 7 seconds after the start of an exposure. They are not always present, but are seen oftener under light than under heavy g forces. They occur oftener and earlier in the monkey than in the dog. The waves suggest a vasomotor or carotid sinus reaction to carotid arterial pressure reduction by acceleratory forces.

There is a reciprocal relationship observed between heart rate and carotid arterial pressure in the monkey under acceleratory forces. In the dog and cat the rate-pressure relations were variable. The Marey law or principle appears to be limited in application, therefore, to primate forms and to certain areal changes in the individual organism. It may be restated as follows: The heart rate usually varies inversely and specifically in relation to carotid sinus pressure changes in the primate animal.

This constitutes a nicely-balanced homeostatic reaction of specific survival value; it is discussed on the basis of its relation to the assumption of the upright position. A similar but less uniform response—probably not yet fully developed—is present in lower mammalian forms.

There were many evidences of the more marked effect of negative acceleratory forces on the organism. a) While heart rate increases were observed to follow arterial pressure decreases by about 3 seconds in positive g tests, under negative accelerations the rate-pressure changes were nearly simultaneous. b) The quickest rate and pressure responses occurred when negative g exposures were imposed on an animal. c) Under equal (but oppositely directed) g forces, heart-rate and carotid-pressure changes were considerably greater under negative than under positive

accelerations. d) Arterial pressure changes were more irregular, also, in negative *g* tests. These and other considerations emphasize the more serious nature of stresses directed toward the head end of the animal.

Pressure levels in the vena cava increased 50 to 100 per cent and in the jugular vein fell to 50 per cent below normal under forces of 1 to 4 *g* for 10 seconds.

Arterial pressure changes under similar accelerations were greater in the dog than in the monkey. A physiological advantage of 0.5 to 1 *g* was shown by the monkey on the basis of blood pressure values in both the carotid and femoral vessels. From other results also noted above, it is clear that a distinct functional advantage is possessed by the primate over lower mammalian forms.

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ESTIMATION OF RELATIVE CORPUSCLE AND SERUM VOLUMES IN BLOOD BY VARIOUS APPLICATIONS OF THE DILUTION PRINCIPLE¹

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IT HAS long been recognized that centrifugal methods do not completely separate blood corpuscles from plasma (1-7). However, attempts to measure the amount of fluid remaining in the sediment have yielded results ranging from 2 to 10 per cent of the packed cell volume (2, 6, 8-14). Most frequently, the estimate has been based on some application of the principle of serum or plasma dilution. Since the divergence of results cannot be explained by variations in technique of centrifugalization, the relative reliabilities of the various methods of applying the dilution principle may reasonably be questioned.

By an extensive series of observations (15) the authors assayed the experimental errors encountered when the dilution principle was applied to known volumes of serum. In such experiments, the possibility of fluid exchange between cells and serum was eliminated by removal of the cells before dilution of the serum, so that the results depended chiefly if not entirely on the accuracy of the measurements involved. Among 10 method variations, the mean errors of estimate were from 3.4 to 8.8 per cent of the true serum volume, i.e. equal to or greater than the error usually ascribed to the centrifugal method. Further, the scatter was so great as to destroy confidence in the results of any single determination, and indicated that such methods were not well adapted to precise serum volume estimation.

The present study extended the investigation to whole blood. Relative corpuscle and serum volumes were estimated in samples of defibrinated beef blood by 12 technically different applications of the serum dilution principle. The results were then compared with those obtained on the same samples by centrifugalization.

METHODS

The principle of dilution involves the measurement of some serum constituent before and after the addition, to a known volume of blood, of a known volume of some suitable diluting fluid. From the concentrations of index material before and after dilution, the original serum volume may be computed by substitution in the equation:

$$V = \frac{RC_2}{C_1 - C_2} \quad (1)$$

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where V represents the original or 'true' serum volume, R is the volume of diluent, and C_1 and C_2 represent the concentrations of the index material before and after dilution, respectively. A variation on this basic relationship is:

$$V = \frac{R(G_2 - G_s)}{G_1 - G_2} \quad (2)$$

where V and R have the same significance as in equation (1), G_1 and G_2 are serum specific gravities before and after dilution, respectively, and G_s is the specific gravity of the diluting fluid.

In these experiments, the following fundamental measurements on serum formed the bases of the dilution procedures: specific gravity by copper sulfate and falling drop methods; total nitrogen; heat-coagulable serum solids; and concentration of the dye T-1824. From certain of these measurements, the following additional indices were derived: serum protein concentration from nitrogen determination and from specific gravity by both methods; serum solids from specific gravity. The diluting fluid was 0.9 per cent solution of sodium chloride, added to blood in the proportion of 1 part to 2. The dye, in 0.5 per cent solution, was added to blood without further dilution. In addition to the above methods, dyed and undyed blood were centrifugalized for one hour at $1400 \times G$, and the amount of fluid trapped in the sediment was estimated by measuring the concentration of dye and of serum solids, respectively, in a saline washing of the sediment.

For convenience of presentation, the methods were numbered as follows:

Method 1. Centrifugalization in a Daland (16) type hematocrit at approximately $7500 \times G$ (17) until the columns of sediment showed no further shrinkage. This method was used as a reference standard.

Method 2. Specific gravity of serum by the copper sulfate method (18), consecutive test solutions of copper sulfate differing in specific gravity by 0.0005.

Method 3. Specific gravity of serum by a modification of the falling drop method of Barbour and Hamilton (19).

Method 4. Serum protein concentration (P) from nitrogen determination: $P = 6.25$ (total nitrogen - non-protein nitrogen).

Method 5. Serum protein concentration computed from specific gravity (G) by the copper sulfate method: $P = 319(G - 1.0055)^2$

Method 6. Serum protein concentration computed from specific gravity by the falling drop method: $P = 312(G - 1.0058)$.

Method 7 (see also *method 8*). Concentration of heat-coagulable, chloroform insoluble, water insoluble serum solids (HCS) in a saline washing of sediment obtained by centrifugalization at $1400 \times G$ for one hour. The technique of washing the corpuscles was essentially that recommended by Maizels (14).

Method 8. Concentration of HCS (20) before and after simple blood dilution. Small, carefully measured volumes of serum were introduced into special, weighed tubes. These were rotated

² In another series of experiments, involving some 200 separate comparisons, linear correlation was established between serum specific gravity and serum protein, as demonstrated by Moore and Van Slyke (22). The equations for *methods 5* and *6* represent regression lines based on this data. These equations have slightly different constants due to a consistent small difference in specific gravity as measured by the copper sulfate and falling drop methods. The equations for *methods 9* and *10* were similarly derived. Equations for the individual correlations were utilized so as to give each indirect method its maximum accuracy.

in an oven at 108° C. so that the coagulated material formed a thin, uniform film on the wall. After 20 minutes of such heating, the films were extracted twice with chloroform and four times with distilled water. The final washings gave no visible precipitate with silver nitrate. The washed films were then dried to constant weight in the oven, again at 108° C.

Method 9. HCS computed from specific gravity by the copper sulfate method: $HCS = 359(G - 1.0061)$.

Method 10. HCS computed from specific gravity by the falling drop method: $HCS = 345(G - 1.0060)$.

Method 11. Total serum nitrogen by a macro-Kjeldahl technique (21).

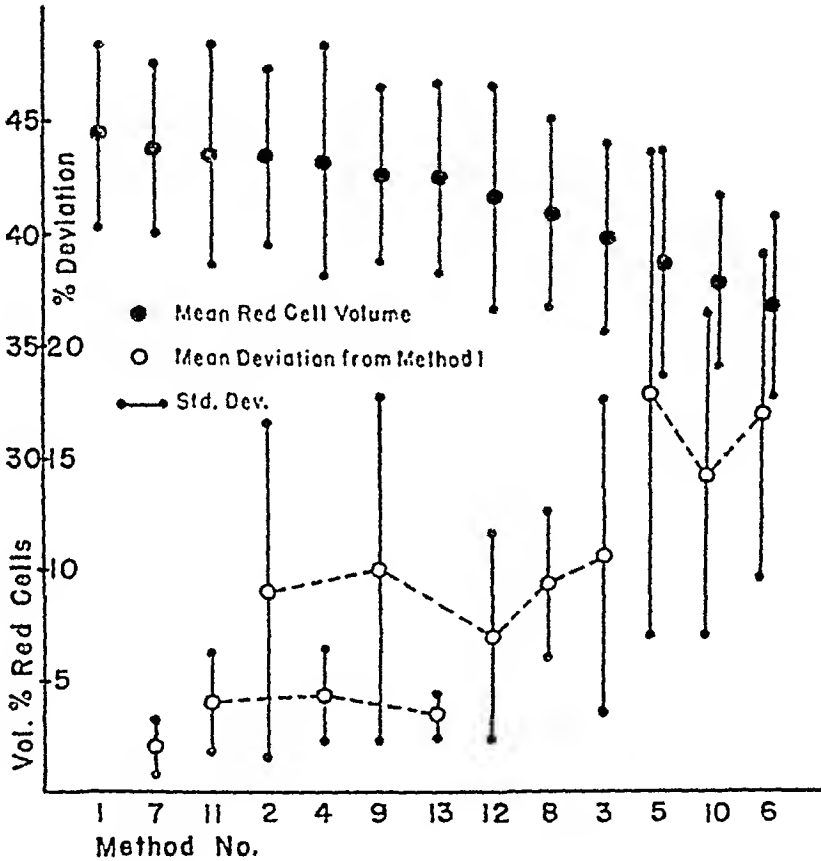


Fig. 1. RELATIVE RED CELL VOLUMES obtained by centrifugal and dilution methods. Methods are designated by number (see text) and are arranged in descending order of mean red cell volume. Broken lines connect members of statistically homogeneous groups among the deviations from method 1.

Method 12. Serum concentration of T-1824, measured at 625 mμ with a Coleman Universa spectrophotometer, using undyed serum from the same blood as a blank. One-tenth ml. of a 0.5 per cent solution of the dye was added to 50.0 ml. of blood. The standard consisted of 0.1 ml. of dye solution in 25.0 ml. of serum from the same blood sample. Dyed serum and blank were diluted with 9 volumes of 0.9 per cent sodium chloride solution to reduce the optical density.

Method 13. Concentration of T-1824 in a saline washing of sediment from blood dyed as in method 12 and centrifugized as in method 7.

RESULTS

The results are summarized in the accompanying table and figure.

Figure 1 shows, by means of closed circles, the mean percentage volume of corpuscles for each method and, by means of open circles, the mean differences for indi-

vidual samples between the centrifugal hematocrit value (*method 1*) and those obtained by the various dilution procedures. This latter information is developed more fully in table 1.

With regard to relative corpuscle volumes, the means for all dilution methods were smaller than that for the conventional hematocrit. The standard deviations were all of the same order of magnitude, indicating favorable distribution of results for general comparisons. The difference from *method 1* approached statistical significance with *method 8*, and was highly significant for the methods with still lower means. The differences between the mean for the centrifugal method and that for the dilution methods varied from 1.4 (*method 7*) to 16.9 (*method 6*) per cent of the mean centrifugal hematocrit. It is obvious that results obtained by dilution methods may differ widely for different technical approaches.

TABLE 1. DEVIATIONS OF RELATIVE RED CELL VOLUMES ESTIMATED BY DILUTION METHODS FROM THOSE OBTAINED BY THE DALAND HEMATOCRIT

METHOD	NO. OF OBS.	MEAN	RANGE	S. D.	S. E.	DIST. OF DEVIATIONS	
						Under 5%	Under 10%
						% of obs.	% of obs.
7, HCS—wash.	15	2.0	0.2—4.5	1.2	0.3	100.0	100.0
13, Dye—wash.	10	3.4	1.6—5.8	1.0	0.3	90.0	100.0
11, N	13	4.0	0.0—9.2	2.2	0.6	69.2	100.0
4, P—N	13	4.3	1.0—9.4	2.1	0.6	69.2	100.0
12, Dye	13	7.0	0.0—17.7	4.7	1.3	23.1	76.9
2, CuSO ₄	22	9.0	0.6—25.4	7.5	1.6	45.5	63.7
8, HCS	18	9.3	2.2—16.5	3.3	0.8	11.1	77.8
9, HCS—CuSO ₄	22	10.0	0.7—25.8	7.7	1.6	31.8	59.1
3, FD	23	10.6	0.4—31.1	7.1	1.5	26.1	43.5
10, HCS—FD	23	14.3	2.8—37.0	7.2	1.5	8.7	21.7
6, P—FD	23	16.9	5.1—39.2	7.2	1.5	0.0	17.4
5, P—CuSO ₄	22	17.9	2.8—45.2	10.8	2.3	4.5	36.4

The average differences, for comparisons on individual samples, between centrifugal and dilution procedures varied with the method from 2.0 to 17.9 per cent of the centrifugal value. The dilution methods seemed to fall into three groups, covering deviation ranges from 2.0 to 4.3 per cent, 7.0 to 10.6 per cent, and 14.3 to 17.9 per cent, respectively. This empirical grouping was confirmed in general by analysis of variance, the results of which are indicated by the broken lines in the lower half of figure 1, and by the divisions of table 1. The lowest group, which was also significantly the most consistent, included both technical variations wherein actual sediment was washed (*methods 7* and *13*). Also, *methods 7* and *13* were the only dilution procedures which yielded no deviations greater than 5.0 per cent. Next in order, still within the first group, were the methods in which total nitrogen (*method 11*) and protein concentration derived from total nitrogen (*method 4*) were used as index measurements. At the other extreme, with high orders of deviation, were methods wherein

the index concentrations were derived indirectly from measurements which, themselves, could be used for the purpose. For example, in *methods 5 and 6*, protein concentration was derived from specific gravity by copper sulfate and falling drop methods, respectively.

The distribution of deviations from the centrifugal value for each of the dilution procedures is also indicated in table 1. The scatter was least for methods with small mean deviations, significant grouping about the mean being confined almost entirely to methods with mean deviations under 10.0 per cent.

DISCUSSION

The results indicated clearly that various applications of the dilution principle are not equivalent, either in the general magnitude of the estimates obtained or in consistency. A rough correlation between degree of scatter and level of deviation was the only hint afforded by the data as to the probable range of real differences between 'true' red cell volumes and conventional hematocrit results. A further point in the same connection was that, while only one method showed all deviations less than 5.0 per cent, all were less than 10.0 per cent for four of the methods, and more than half of the individual differences were less than 10.0 per cent for four additional methods (table 1). If one may provisionally infer on such a basis that the true differences probably were somewhat less than 10.0 per cent, then all dilution methods tested were capable of giving reasonable results part of the time, but certain ones were just as apt, or even more likely, to yield highly erroneous and completely misleading results on any given single trial. The same point was illustrated in a striking manner in the control series previously reported (15).

While it may be safely assumed that all centrifugal methods yield cell volumes which are too high, the results did not justify the selection of any one of the dilution methods as estimating the 'true' cell or serum volume. Certainly, the indirect methods, wherein the index measurements were derived secondarily by mathematical treatment of observed data, proved generally unreliable (e.g. *methods 5, 6, and 10*). Such methods were included in the study only because some of them have appeared in the literature. Even without these, there still remained several dilution procedures among which any single choice would have been arbitrary.

Experience in applying the dilution principle, both in the present and in previous studies, suggested that the unfavorable distribution of errors encountered, as well as the wide divergence in results from the literature, might be explained in part by the nature of the mathematical relationships involved in the estimate. Analysis of equation (1) revealed that relative or percentile errors in the measurement of index materials become magnified in the result; that if the volume of diluent (R) is chosen to approximate the expected serum volume (V), the percentile error in estimating V is about twice that of measuring either of the index concentrations C_1 or C_2 ; that as R decreases below V , the ratio of errors in V to errors in C_1 or C_2 mounts very rapidly, while, conversely, as R increases beyond V , the ratio diminishes very slowly, approaching only at infinite dilution a limit where percentile errors in V and in C_1 or C_2 are equivalent. For any given value of R , the percentile error produced in V by a given percentile error in C_1 is numerically equal to that which results from a

like error in C_2 , but is of opposite sign. Hence, equal percentile errors of like sign in C_1 and C_2 would neutralize each other, but errors of opposite sign would become additive in V . If, broadly speaking, errors of measurement (C_1 or C_2) are normally distributed, neutralization can be expected in only half the trials, and even this neutralization is incomplete unless the percentile errors in C_1 and C_2 are equal. When addition of errors occurs (also in approximately half the trials), the situation is rendered even more unfavorable by the magnification referred to above. As an example, assume that $R = V$ and that there is a $+5$ per cent error in measuring C_1 , a -5 per cent error in C_2 . The resultant error in estimating V would approach -20 per cent. With regard to absolute (as contrasted with percentile) errors, those encountered in C_2 produce larger errors in V than do numerically equal errors in C_1 . In general, the implications of equation (2), with specific gravity as the index, are similar to those just described, the magnification of errors being even greater than for equation (1). Thus, when $R = V$, and G_1 is in the range usually encountered, the percentile error in estimating V is about two and one half times that of measuring G_1 and approximately three times that of measuring G_2 . When, on the other hand, a known quantity of dye is added to serum, the percentile errors of serum volume estimate and of dye measurement are numerically equal.

With regard to dilution procedures, then, three facts are obvious: *a*) small volumes of diluent should be avoided, while amounts greatly in excess of the expected serum volume have no serious advantage; *b*) precise estimates of serum volume by the dilution principle require the utmost accuracy in measuring the index concentrations; *c*) the relationships are such as to account readily for very erratic serum volume estimates even with fairly good analytical methods. The discrepancies reported here and elsewhere are probably due in large measure to variations in precision among the methods employed. Undoubtedly, some of the analytical procedures can and will be improved to meet more nearly the exacting requirements imposed by the dilution equations. The present study included methods and modifications which have been applied to the problem by other investigators (8, 11-14).

The dilution principle and factors based upon it are widely used for estimating 'true' relative corpuscle and serum volumes in blood, or for correcting conventional hematocrit results. The data presented certainly inspire no confidence in the results of a single determination by any of the dilution methods tested. Similarly, on a statistical basis, any one of several hematocrit correction factors might have been chosen. While routine correction must be less hazardous than the employment of dilution methods alone (i.e., without centrifugal control), results so obtained are not considered superior to uncorrected centrifugal values unless the correction factor can be more precisely defined.

SUMMARY

Relative corpuscle and serum volumes were estimated in samples of defibrinated beef blood by 12 technically different applications of the serum dilution principle, and the results were compared with those obtained on the same samples by means of a Daland type hematocrit.

Mean differences between centrifugal and dilution results varied with the method

from 2.0 ± 1.2 to 17.9 ± 10.8 per cent of the packed cell volume. The results did not permit choice of any one dilution procedure as estimating reliably the 'true' cell or serum volume. The wide dispersion of results obtained by dilution methods depends in part upon the nature of the mathematical relationships employed in the calculations. Practically, most dilution procedures are not well adapted to precise serum volume estimates.

Correction of conventional hematocrit results by a constant factor based on dilution methods is not justified by present data.

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STUDIES IN INTRACARDIAC ELECTROGRAPHY IN MAN.

III. DISPLACEMENT OF THE CARDIAC PACEMAKER¹

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IN A general way an electrode within the right atrium records auricular deflections which are *a*) predominantly downward when high in the atrium, as confirmed roentgenologically; *b*) biphasic (initially upward) when in the middle portion of the right atrium; and *c*) predominantly upward when low in the right atrium (1). These variations in form correspond to the fact that in the first instance the wave of auricular depolarization recedes from the electrode, in the second instance the wave of depolarization approaches then recedes from the electrode and in the third instance the impulse approaches the electrode. It was suggested (2) that utilization of the technique of intracardiac electrography and the known facts regarding the intra-atrial potentials might provide more precise information regarding displacement of the auricular pacemaker.

Vagal stimulation through the oculo-cardiac reflex may cause the P-wave to decrease in height, become isoelectric or inverted (3). In a large proportion of normal young persons, Wilson (4) was able to displace the pacemaker to the auriculo-ventricular node by ocular stimulation during the period between the hypodermic injection of atropine and the appearance of its maximal effect. Vagal stimulation through the carotid sinus reflex may likewise cause the P-wave to decrease in height, become isoelectric, notched or inverted (5, 6).

METHOD

In the present study 16 individuals without heart disease ranging from 19 to 65 years of age (av. 33 yr., mean 28 yr., 12 male and 4 female) were catheterized by essentially the same technique as that described in a previous communication (1). Unipolar intracavity electrograms were obtained from high, mid and low positions in the right atrium at slow (25 mm/sec.) and fast (75 mm/sec.) speeds and 'spot films' were exposed just before or after each tracing. Care was exercised that the 'spot film' was taken during the same respiratory phase in which the tracings were taken. With the Sanborn 'Tribeam' vacuum tube type of electrocardiogram³ simultaneous tracings were taken usually of VF, occasionally of VL, VR or V₁. At

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each level additional tracings were taken before, during and after stimulation of the right carotid sinus (rarely the left carotid sinus as well) and of the right (the left in only one instance) eyeball. The entire procedure was repeated during the period elapsing between about 8 and 20 minutes following the subcutaneous injection of 1.2 mg. of atropine sulfate.

Pseudo-Displacement of the Pacemaker. When the left antecubital vein was catheterized and the catheter entered the thorax through the left axilla, right carotid sinus stimulation produced either a very inconspicuous change or no change whatever in its position. This is illustrated in a subject catheterized through the left antecubital vein. The roentgen-ray films were exposed before and during right carotid sinus stimulation by a double-exposure technique. At none of the three positions did this procedure change the position of the catheter significantly. Left carotid sinus stimulation, on the other hand, regularly produced slight but definite changes in the position of the catheter in the atrium. It is possible then that homolateral neck pressure may alter the position of the electrode, the position of the heart remaining fixed and, thus, produce the false appearance of displacement of the pacemaker.

The appearance of displacement of the cardiac pacemaker may be simulated in another way. The relationship of the pacemaker and the electrode may vary as a result of respiratory changes in the position of the heart in the thorax, the electrode remaining in a fixed position. The degree of this change varies from individual to individual, apparently depending upon the degree of diaphragmatic respiration. In an extreme example, deep inspiration can alter the position of the heart so that the tip of the catheter, initially at a low atrial position, now lies opposite the mid-portion of the atrium. Since the electrical potentials recorded at the electrode may vary not only because of a varying site of origin of the impulse in relation to a fixed electrode (true displacement) but also because of a change in the position of the electrode in relation to a fixed pacemaker (pseudo-displacement), the position of the heart must remain independent of respiration.

It is well known that respiratory (7) as well as cardio-inhibitory and depressor reflexes may be initiated by carotid sinus or ocular stimulation. Pseudo-displacement of the cardiac pacemaker produced in this way is illustrated in figure 1 taken low in the right atrium of another subject 9 minutes after the subcutaneous injection of 0.9 mg. of atropine sulfate. With right carotid sinus stimulation there was a smooth progressive change in the form of the auricular potentials (upper tracing) which were initially biphasic, but were directed entirely downward by the sixth complex, subsequently returning to a biphasic complex. Simultaneously recorded lead V_1 (lower tracing) showed no change in the form of the P-wave corresponding to the changes in the intra-atrial potentials. The change in the form of the auricular complex can be accounted for by an uncontrolled inspiration during carotid sinus stimulation so that with inspiration the site of impulse formation was drawn down to the tip of the catheter. In this curve it is to be noted that the heart accelerated rather than slowed during carotid sinus stimulation.

Because of the difficulty in interpreting tracings of this sort with uncontrolled respiration, carotid sinus or ocular stimulation was performed in most of these subjects while the breath was held in deep inspiration. All but a few were thus able to

inhibit any respiratory reflexes which might have been induced by the procedure. With these two provisions then, namely, that the side of the neck opposite the catheterized side was stimulated and that the procedure was carried out while the patient held his breath in maximal inspiration, it was possible to proceed on the assumption that any consistent changes in the direction of the auricular complex might properly be attributed to displacement of the cardiac pacemaker. It must be emphasized, however, that despite all these efforts to insure a constant relationship between heart and catheter, minor variations were still possible because of the location of the tip of the catheter free in the atrial cavity.

RESULTS

Of 16 subjects tested, true displacement of the cardiac pacemaker was produced by carotid sinus or ocular stimulation in 9 individuals, pseudo-displacement in 2 and no displacement in 4 individuals, in spite of well-marked slowing. In one subject we are not certain whether true displacement occurred. One tracing in this subject showed the gradual development and recession of changes in the auricular complex, but there was no co-incident changes in the form of the P-wave in simultaneously recorded lead VF. This may possibly be attributed to the fact that so little change was thus produced in the position of the pacemaker that it was not projected into lead VF. Since she was able to maintain a steady unwavering deep inspiration, a spontaneous shift in the relative positions of the electrode and the pacemaker seems unlikely.

Generally the displacement began during the period of stimulation and subsided at or shortly after its release. Occasionally the displacement did not begin until after the end of the period of stimulation or, at times, it began during the latter part of the period of stimulation and outlasted it for varying periods. In general, the displacement was much more readily induced by ocular than by carotid sinus pressure, but this may be attributed to our curtailment of the period of carotid sinus stimulation to five seconds or less. We were much more apt to maintain ocular pressure for several seconds longer.

In one individual among the first 8, displacement was as easily induced before as after the administration of atropine. In all of the others the effect was noted only in the period between 8 and 20 minutes or so after the subcutaneous administration of 1.2 mg. of atropine sulfate. For this reason the preliminary period of observation without atropine was omitted in the latter 8 subjects.

Seven of the 9 individuals in whom displacement was produced showed simultaneous slowing of the heart rate, 2 showed no slowing. In only one case were we successful in producing atrio-ventricular nodal rhythm; auricular standstill with nodal escape was produced in one patient.

ILLUSTRATIVE OBSERVATIONS

Figure 2 illustrates true displacement of the pacemaker beginning abruptly with the auricular complex just preceding the release of right ocular pressure. The auricular complex, having been biphasic until this point, suddenly became a monophasic complex entirely directed downward. With release of ocular pressure there

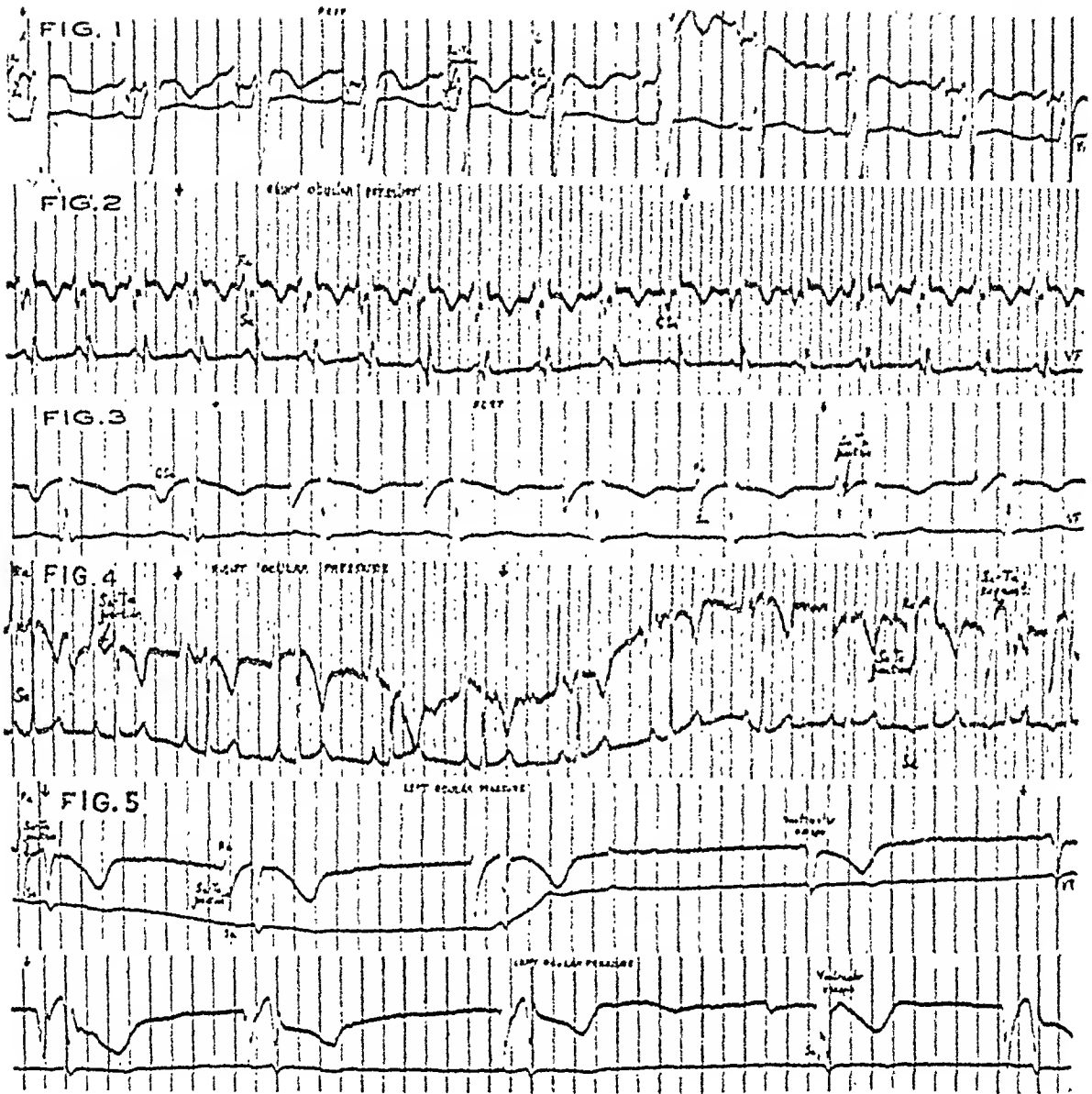


Fig. 1. PSEUDO-DISPLACEMENT OF CARDIAC PACEMAKER. Nine minutes after the subcutaneous injection of 0.9 mg. of atropine sulfate. Uncontrolled inspiration produced by right carotid sinus stimulation. Smooth progressive change from biphasic (Ra-Sa) auricular deflection in 1st complex to monophasic (QSa) auricular deflection in 6th complex, recorded at low auricular level. (*Upper tracing*) The movement of the upper curve toward the upper edge of the paper is an artefact coinciding with the release of carotid sinus pressure. No corresponding changes in P-wave of simultaneously recorded lead V₁ (*lower tracing*). Note coincident depression of Sa-Ta likewise explicable as result of change in level of electrode.

Fig. 2. TRUE DISPLACEMENT OF PACEMAKER. Mid-atrial potentials (*upper curve*) paired with VF (*lower curve*). Right ocular pressure 14 min. after the subcutaneous injection of 1.2 mg. of atropine sulfate. Following a period of slowing there is an abrupt change in the form of the auricular complex just preceding the release of ocular pressure from an equiphase (Ra-Sa) to a monophasic (QSa) form, indicating migration of pacemaker from a point high in atrium to a mid-point in the atrium. Note smooth, progressive return of auricular complex to original form and correlative abrupt decrease in amplitude of P in lead VF with shortening of P-R interval from 0.14 to 0.11 sec., followed by gradual reassumption of original form of P-wave and return of P-R interval to its original duration. The effect of displacement outlasts the period of stimulation.

Fig. 3. TRUE DISPLACEMENT OF PACEMAKER. High auricular potential (*upper curve*) paired with lead VF (*lower curve*), recorded 9½ minutes after 0.9 mg. of atropine sulfate intravenously. Arrows indicate onset and offset of right carotid sinus stimulation. There is a progressive relative

was a smooth progressive return to a biphasic auricular complex over the next four beats. Corresponding to these changes in the intra-atrial potentials the P-wave of simultaneously recorded lead VF suddenly decreased in amplitude, then over the next four beats showed a smooth progressive return to its original form, while the P-R interval suddenly shortened from 0.14 to 0.11 second, then gradually returned to its original duration. The changes recorded seem to correspond to a migration of the cardiac pacemaker from a point high in the right atrium to one at mid-atrial levels, that is, approximately from the position of the tip of the catheter in figure 6A to that in figure 6B, the level at which the upper curve of figure 2 was obtained. In the frontal projection recorded by the 'spot films' this corresponds to a distance of about

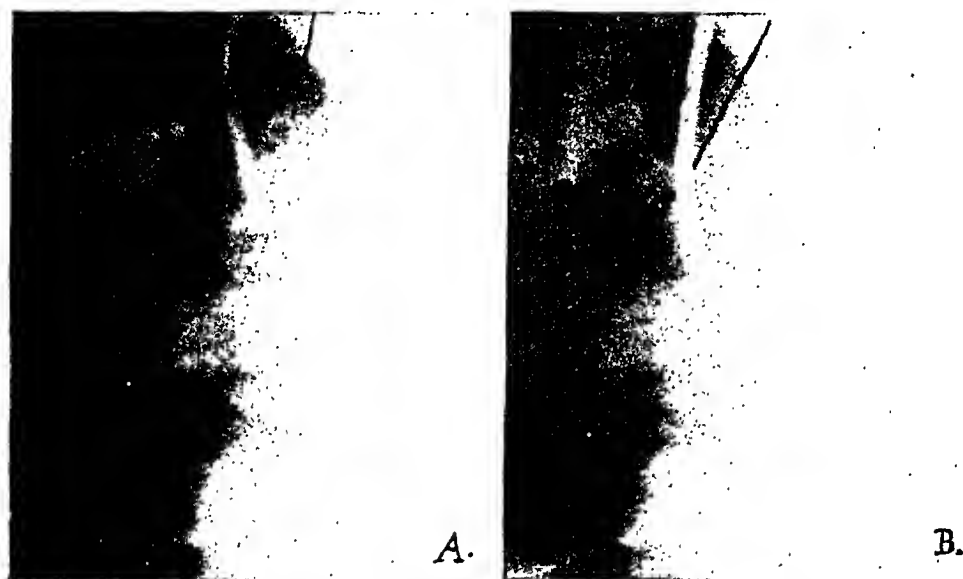


Fig. 6A. ROENTGENOGRAM FROM SUBJECT with electrode high in right atrium where QSa deflections were recorded indicating that normal site of impulse formation was close to the tip of the catheter. B. Roentgenogram showing level at which upper curve of fig. 2 was obtained and toward which level the site of impulse formation migrated on ocular pressure. This indicates about 3 cm. of dislocation of the pacemaker as measured in the frontal plane.

3 cm., which is probably a minimum since the posterior wall of the atrium has the shape of a hemisphere rather than a flat surface. With the catheter free in the auricular cavity, however, so many things can happen that it is impossible to venture a definitive interpretation of the findings.

increase in the height of Ra coinciding with an irregular slowing of the pacemaker, movement of the Sa-Ta junction closer to the isoelectric line, and decrease in the height of P VF to an isoelectric position. No obvious change in P-R interval.

Fig. 4. DISPLACEMENT OF PACEMAKER to the auriculo-ventricular node 4 sec. following ocular pressure. Patient had received 1.2 mg. of atropine sulfate subcutaneously 16 min. previously. Slight slowing during ocular pressure. Abrupt displacement of pacemaker to point near intraatrial electrode which was about three fourths of the distance from the superior to the inferior vena cava, indicated by change from biphasic auricular potentials, with larger component directed upward, to largely monophasic downward auricular potentials as revealed in auricular cavity and reversal of P in lead VF. Apparent speeding up of final beat is due to hesitation of drum of camera. Note depression of Sa-Ta junction and elevation of Sa-Ta segment with new pacemaker.

Fig. 5A (upper). DISPLACEMENT OF PACEMAKER with slowing then auricular standstill and ventricular escape. Upper curve, mid-auricular potentials; lower curve, unipolar right arm potentials (VR). Left ocular pressure. B (lower). Same subject at high auricular levels.

Figure 3 shows exactly the same sort of change in another subject viewed from an opposite vantage point. Here when the pacemaker was displaced, the impulse approached rather than receded from the electrode. Patient had received 0.9 mg. atropine sulfate $9\frac{1}{2}$ minutes previously. The upper curve was obtained high in the right atrium, the lower again represents lead VF. Right carotid sinus pressure produced slight slowing coinciding with displacement of the pacemaker. In the second complex, which just precedes the onset of carotid sinus stimulation, the auricular complex was directed entirely downward, indicating that at that time the site of impulse formation was near the electrode. In the sixth complex the auricular potentials were almost equiphasic indicating that at this moment the impulse first approached then retreated from the recording electrode. At the same time the P-wave of lead VF became low and biphasic. Measurement of the P-R interval was impossible in VF while in the intracavity tracing it showed no change.

Figure 4 shows the potentials in a low-mid position of the right atrium of another subject 16 minutes after the subcutaneous injection of 1.2 mg. of atropine. During right ocular pressure the heart slowed but the form of the auricular complex, aside from some artefacts, showed no change. At about four seconds following the release of ocular pressure there was a sudden change in the auricular potentials, which were now practically entirely directed downward. That these were not extrasystoles, stimulated by the contact of the tip of the catheter with the atrial wall, is indicated by the fact that each began with a minute upward (Ra) deflection; if these were induced premature beats one would expect the auricular deflection to begin abruptly downward. At the same time, the Sa-Ta junction was depressed and the Sa-Ta segment elevated. The new pacemaker was more rapid than the original sino-auricular pacemaker. Coinciding with the change in the intracavity potentials, P_{VF} became inverted, though of smaller magnitude, but the P-R interval did not shorten. The new rhythm center, therefore, seemed to reside in the auriculo-ventricular node. From the reversal in direction of the P-wave one may suspect, however, that the impulse now took an almost diametrically opposed course, changing from above-downward to below-upward. Measurement of 'spot films' showed that in frontal projection the new center of impulse formation was at least 4.5 cm. from the probable location of the original pacemaker. This again is a minimum figure. The degree of displacement is apparently beyond the traditional confines of the sino-auricular node.

Figure 5A shows the potentials at mid-atrial levels (upper curve) and the right shoulder (VR) of a 25-year-old seaman 21 minutes after 1.2 mg. of atropine were injected subcutaneously. The first complex after the beginning of the electrogram was equiphasic but the second and third showed small Ra, deep Sa and depressed Sa-Ta segment. Following this there was auricular standstill until ocular pressure was released. The fourth and fifth complexes were ventricular escape beats. In this case the displacement seemed to correspond approximately to a change in the site of impulse formation from high to mid-auricular levels. With displacement there was no measurable decrease in auriculo-ventricular conduction time as measured in the cavity potentials.

Figure 5B shows the potentials recorded high in the right atrium of the same

individual. Left ocular pressure slowed the heart without displacing the pacemaker. The fourth complex showed a short interval between the intracavity auricular and ventricular deflections, due to ventricular escape. If this were an auriculo-ventricular nodal beat, one would expect the chief auricular deflection to be upright, indicating passage of the impulse *toward* the electrode high in the atrium, rather than downward, as it was here, indicating persistent origin of the impulse near the electrode and its spread *away from* the electrode.

DISCUSSION

It is quite generally believed that the cardiac pacemaker resides in a club-shaped anatomical structure, the sino-auricular node, situated high in the right atrium and measuring about 1.5 cm. in its greatest length. It is also believed that the pacemaker may wander from the head, to the body or tail of this sino-auricular node or to a position in the upper, middle or lower parts of the auriculo-ventricular node (8). However, the possibility of the pacemaker wandering to portions of the atrium intervening between the sino-auricular and auriculo-ventricular nodes has not been mentioned. The facts presented in this study are consistent with the possibility of displacement of the pacemaker beyond the accepted limits of this structure high in the right atrium. It is felt that the method employed is not precise enough to prove or disprove displacement within such a small area, but it has been capable of recording apparent gross displacement to mid-auricular levels (figs. 2, 3, 5) and to the auriculo-ventricular node (fig. 4).

In the experimental animal strong vagal stimulation produces marked shortening of the slow deflections indicating auricular repolarization (9). The first observable effect is a deformity of the segment which immediately follows the rapid depolarization wave, that is, of the Sa-Ta junction. In many cases in the present study, the Sa-Ta junction became depressed as Ra decreased in height (figs. 4 and 5) and correspondingly the Sa-Ta junction became displaced toward the isoelectric line as Ra increased in height (fig. 3) but in one case (fig. 2) the Sa-Ta junction moved toward the isoelectric line as Ra decreased. The significance of such deviations of the Sa-Ta junction in man is not clear. In a previous communication (1) it was pointed out that such changes may vary with the site of the electrode in the right atrium. In the present study such variations were, insofar as possible, excluded. It seems possible, then, that vagal stimulation, in addition to displacing the cardiac pacemaker, may alter the speed of the repolarization process in the auricles.

The fact that with displacement of the pacemaker the P-R interval shortened very little (fig. 2) or not at all (figs. 3A, 4, 5A) corresponds to the well known fact that conduction in the auricular musculature is very rapid and hardly represented in the P-R interval, while the bulk of the auriculo-ventricular conduction time is consumed in the atrio-ventricular node and bundle.

Wilson (4) produced nodal rhythm in a large proportion of young normal individuals under virtually the same conditions as the present experiments. Our failure to duplicate these results in all but one individual may, in part, be due to the greater average age of the patients available for this study. It may also be explained in part by our limitation of the period of carotid sinus stimulation to four or five seconds,

beyond which, particularly in the described experimental set-up, we did not feel it safe to continue.

Although in most individuals displacement was accompanied by cardiac slowing, in a few, displacement occurred without slowing. This suggests that displacement of the pacemaker may be independent of the mechanism responsible for reflex slowing of the heart. In 2 subjects the rate of the new pacemaker was more rapid than that of the sinus node. The nodal rhythm induced by Purks (6) in one patient with carotid sinus stimulation was more rapid than the original sinus rhythm and the rhythm persisted after the cessation of carotid sinus stimulation. In the only instance in which he produced flattening of the P-wave, the heart rate was at first faster then slower than the original sinus rhythm. The conditions of the present study differ from the latter in that these subjects were under the incomplete effect of atropine. It is suggested that since the sinus node is more richly supplied with vagus nerve endings than the auricular musculature proper, vagal stimulation has a greater effect in inhibiting the sino-auricular node than the auricular musculature or the various subsidiary pacemakers in the auricular musculature. During the period between about 8 and 20 minutes after the subcutaneous injection of atropine, the sinus node is not yet accelerated. Inhibition of the sinus node at this time may release a center or series of centers whose rate is more rapid than the sinus node.

SUMMARY

In a group of incompletely atropinized individuals without heart disease, vagal stimulation through the oculo-cardiac or carotid sinus reflex frequently produced displacement of the cardiac pacemaker. A study of intra-atrial potentials in relation to roentgen-ray examination seemed to place the new site of impulse formation halfway down the right atrium. In only one subject was nodal rhythm produced. Coincident with the displacement there was very little or no shortening of auriculo-ventricular conduction time. It is possible that in man the auricular repolarization process may also be altered by vagal stimulation. There is often a downward displacement of the pacemaker when the heart is slowed by vagal stimulation in man as there is known to be in animals.

We are grateful to Dr. Frank N. Wilson for his criticism of the manuscript.

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ELECTROGRAM OF ISOLATED PAPILLARY MUSCLE OF THE CAT HEART¹

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A RECENT review has demonstrated that the theories of various investigators concerning the genesis of the electrocardiogram are far from similar (1). The general position taken by Craib (2, 3) and those following his views (4-6) that valid information is only obtained by considering the heart as a 'dipole' in the center of a 'volume conductor' is opposed by Gilson and Bishop (7). Upon closer study one is struck by the dissimilar and often apparently moribund preparations used to obtain the data upon which these hypotheses are based. It is possible that some of the discrepancies could be resolved if a suitable preparation were more generally employed. The ideal preparation "is one in which there is at least an approach to propagation along a linear and uninjured tissue" (7). The present communication is believed to describe such a preparation together with some initial observations upon it.

The papillary muscles of the cat's right ventricle have proved very convenient for the study of physiological and pharmacological factors affecting the force of contraction of cardiac muscle (8-11). By virtue of their structure they may be isolated easily and, under suitable conditions (10), they contract vigorously upon stimulation for as long as 24 hours. Care in choosing a muscle for study will result in an uninjured portion of mammalian ventricular muscle consisting of about 50 almost perfectly parallel cells from which recordings of contractile and electrical activity may readily be obtained. As details of technique in such experiments have considerable bearing upon the significance of the findings, they are described at length.

METHOD

Under ether anesthesia a cat's heart was removed and an incision made along the border of the right ventricle. Suitable papillary muscles³ were removed with the chordae tendinae at the tip, and part of the ventricular or septal wall at the base still attached. *The papillary muscle itself was not cut or injured in any way.*

The muscle was mounted in a holder, and the chordae tendinae attached, by

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³ During an ancillary study, Mr. S. F. Redo observed that papillary muscles which had a diameter greater than 1 mm. showed central areas of degeneration (a markedly modified staining reaction, loss of striation and poor definition of cell structures) after several hours of contraction. The relative width of normal and degenerated areas was in general agreement with current concepts of the diffusion of oxygen into muscle (12).

means of a fine silk thread, to an isometric muscle lever, the movements of which were recorded by a photokymograph. The base was tied to an adjustable hook. The two silver-silver chloride recording electrodes were Y-shaped and the muscle was placed between the forks of the Y to assure good electrical contact. The upper electrode was adjustable and was always placed on the tendon. The lower electrode was in contact with the lower third of the muscle. The two stimulating electrodes were placed in contact with the fragment of ventricular wall and arranged so that the stimulus passed across the base of the muscle, at right angles to the long axis of the muscle.

The glass chamber surrounding the muscle holder was then filled with a modified Locke's solution (11) to a level of about 1 cm. above the chordae tendinae. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was forced into the bottom of the chamber where a sintered glass plate increased the dispersion. The optimum rate of gas flow was found to be very rapid. The holder was then immersed in a water bath at 37°C., unless otherwise noted. Diastolic tension was adjusted to the lowest tension which resulted in maximum systolic force.

The electrograms were recorded with a direct writing electrocardiograph the push-pull input circuit of which possesses excellent rejection of 60-cycle alternating current interference (Electro-Physical Laboratories 'Cardiotron'). The sensitivity in all cases was adjusted to bring the electrogram to a convenient size (QRS about 1.5 cm. high). By comparison with the 1 mv. calibration pulse the potential of the electrogram was then found to be of the order of 10 mv. The connections were arranged so that negativity at the tendon caused an upstroke of the writing stylus. When it was desired to record the electrical and mechanical activities simultaneously on the photokymograph a strong thread was tied to the base of the writing arm of the electrocardiograph. With the instrument on its side, a small hole in the cabinet permitted attachment of the thread to a stiff isometric lever under considerable tension. The movements of this lever could be recorded in the usual way and were surprisingly faithful reproductions of the writing arm movements.

Although most preparations contracted spontaneously at first, it usually became necessary to stimulate the muscle electrically. This was done with a thyatron stimulator ('Electrodyne') with the stimulus slightly above threshold levels. The polarity of the stimulus was adjusted so that the stimulus artefact appeared in the electrogram as a slight initial downward deflection simulating a Q-wave.

Ten seconds before an electrogram was recorded, the level of fluid in the chamber was lowered to about 1 cm. below the base of the muscle to prevent the short-circuiting of the recording electrodes by the fluid as well as to prevent the introduction of too large a shock artefact into the record.

EXPERIMENTAL

Electrogram Associated with Spontaneous Contraction of the Isolated Papillary Muscle. Of the 60 papillary muscles prepared, 14 were still contracting spontaneously when the first electrograms could be taken (about 4 min. after cardiectomy). Figure 1 presents some of the electrograms associated with these spontaneous contractions. A characteristic electrographic pattern is apparent which consists of an

upright R-wave⁴ followed by an upright T-wave just as seen in the usual limb-leads. Diphasic R-spikes were never obtained. Although it can be seen that the configuration of the T-wave varies, its polarity is consistently upright.

Effect of Oxygen. Although many thin and quickly prepared isolated papillary muscles, whether still contracting spontaneously or requiring stimulation, often manifested a T-wave which was in the same direction as the R-wave, the electrogram of the majority of stimulated muscles when first examined showed the type of T-wave

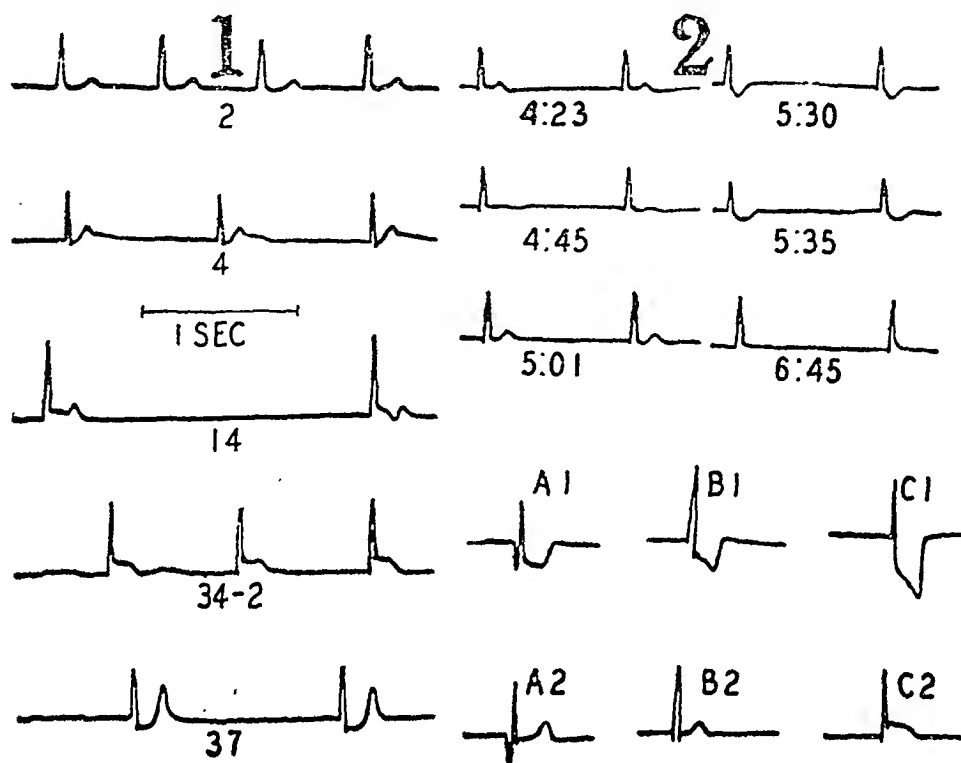


Fig. 1. ELECTROGRAMS ASSOCIATED WITH SPONTANEOUS CONTRACTIONS of papillary muscles. The displaced S-T segments noted disappeared slowly during oxygenation and stimulation, suggesting that there are injury currents in freshly prepared muscles which gradually diminish.

Fig. 2. UPPER RECORDS ARE FROM A SINGLE PREPARATION. 4:23 Control electrogram 95% O₂, 5% CO₂ bubbling through chamber. 4:42 Rate of gassing decreased. 4:45 Electrogram recorded. 4:46 Rate of gassing increased. 5:01 Electrogram recorded. 5:05 Nitrogen substituted for oxygen in the gas mixture. 5:30 Electrogram recorded. 5:32 Oxygen re-introduced into gas mixture in place of nitrogen. 5:35 Electrogram recorded. 6:45 Electrogram recorded. Apparently, the brief period of severe anoxia produced some permanent damage. Contractility was greatly depressed during the periods of anoxia. In the lower portion of this figure are the electrograms from 3 muscles (A, B and C) before adequate oxygenation at 1 and after adequate oxygenation at 2.

customarily associated with myocardial anoxia *in situ*, namely, an inverted or downward deflection. Following a variable period of rapid gassing in the isolation chamber (duration proportional to muscle thickness) the T-wave became upright with recovery of the S-T segment (fig. 2). This suggested so strongly that the direction of the T-wave could be influenced by the oxygenation, not only of the heart as a whole but of the isolated papillary muscle as well, that further experiments were performed.

⁴ Although the letters Q, R, S, and T are employed to designate deflections in the limb-leads they will be applied here to the similar patterns of the papillary electrogram.

In a series of five preparations in which the T deflection was upright, decreasing the rate of flow of the usual gas mixture through the solution decreased the height of the T-wave. When the rate of flow was increased and an electrogram taken a few minutes later the T-wave was found once again to be at its initial height. To avoid possible changes in pH a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide was substituted for the oxygen-carbon dioxide mixture and allowed to bubble through the solution at approximately the same rate. After a period of from 5 to 20 minutes

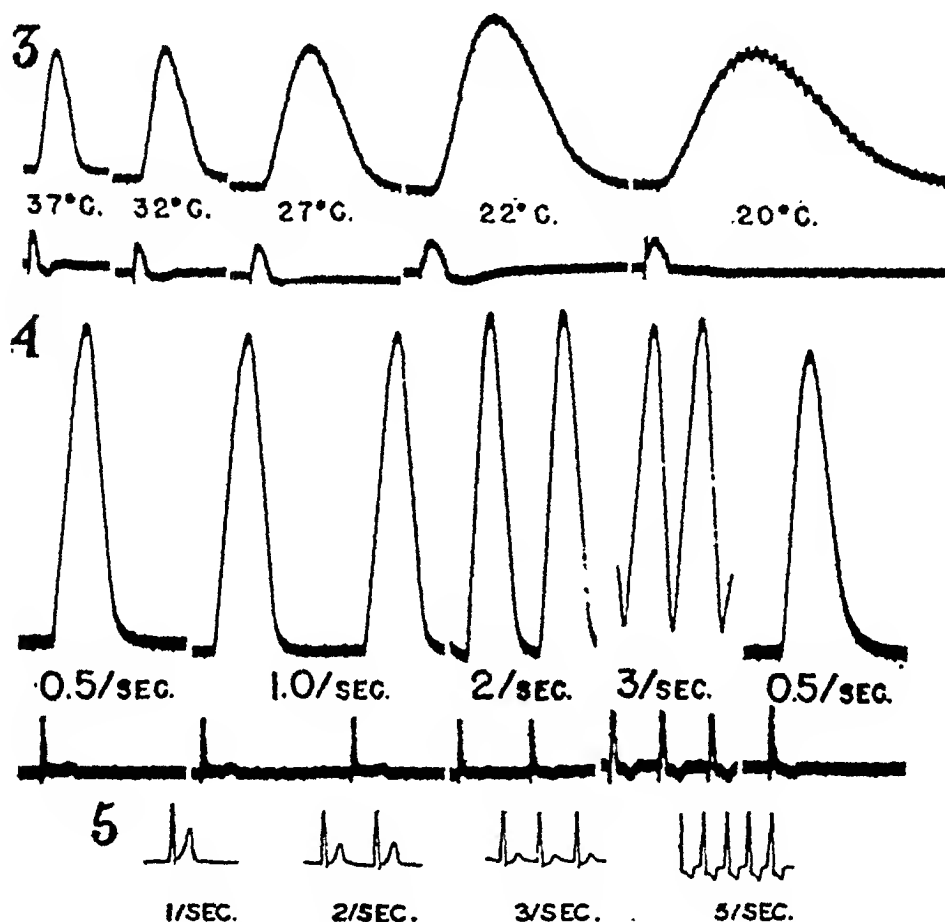


Fig. 3. CHANGES IN THE ELECTROGRAM AND MYOGRAM of a papillary muscle during changes in temperature. There is some indication that the muscle was not in perfect condition at the beginning of the experiment but the results of temperature change are clear cut and have been repeatedly observed. The lower tracing is the electrogram.

Fig. 4. EFFECT OF CHANGES in rate on the electrogram and myogram recorded simultaneously from a papillary muscle. *Upper record: myogram; lower record: electrogram.*

Fig. 5. EFFECT OF CHANGES in the rate of stimulation on the T-wave of the papillary electrogram.

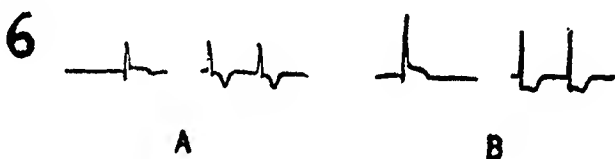
another electrogram was recorded. In each case this electrogram showed an inversion of the T-wave which was generally quite marked. Replacing the nitrogen with oxygen produced a definite return of the T-wave toward the isoelectric line after several minutes. After one-half to one hour of oxygenation the final electrogram once more revealed the T-wave and S-T segment to be upright. These changes are shown in figure 2.

Effect of Temperature on the Electrogram and Myogram. The effects of temper-

ature changes on the character of the electrogram and myogram were examined on five papillary muscles. Records were made at temperatures of 42°C., 37°C., 32°C., 27°C., 22°C. and occasionally at 17°C. when contraction would occur. The rate of oxygenation of the solution bathing the papillary muscle remained unchanged during the entire series of recordings.⁵ Figure 3 shows the electrograms recorded from a single papillary muscle at different temperatures. It is apparent that the duration of the R-wave, the R-T length, the duration of the T-wave, and the time taken for contraction of the muscle all increase 100 per cent for each decrement of 10°C. The increase in the amplitude of contraction with decreased temperature has long been known (13) and apparently the quantitative aspects of the present records agree well with the consideration of energetics outlined recently by Varga (14) for rabbit skeletal muscle.

Effect of Rate Changes. The effect of rate changes was studied on nearly all of the papillary muscles used. All other conditions were unchanged and only the rate of stimulation varied. Control electrograms were taken with the muscle stimulated

Fig. 6. EFFECT OF EPINEPHRINE AND EPHEDRINE on the T-wave of the papillary muscle. In experiment A epinephrine (1:2,000,000) caused rapid spontaneous contractions with the result shown to the right of A. In experiment B ephedrine sulphate (1:50,000—see Krop⁶) caused a rapid spontaneous contraction with inversion of the S-T segment.



once per 2 seconds. Electrograms were then taken with the muscle stimulated at rates of 1, 2, 3 and 5 per second. Following this, another electrogram was usually taken with the muscle stimulated at the control rate of one per 2 seconds. Figures 4 and 5 illustrate the findings at different rates of stimulation. It is evident that the R-T length diminishes as the rate increases and that with increasingly rapid rates of contraction, the T-wave becomes depressed and finally inverted. The 'treppe' observed by Cattell and Gold (15) was regularly noted in vigorous preparations.

Conversely, when preparations which manifested an inverted T-wave at stimulation rates of one per second were stimulated at a rate of once in 30 seconds, the T-wave associated with these isolated contractions was always upright. This emphasizes the concept that relative, as well as absolute anoxia may control the T-wave direction in the papillary muscle as in the total heart.

Tension. Changes in tension within a physiological range, as indicated by slight or no impairment of contractility, were without effect on the character of the electrogram. Overstretching produced a marked depression of the S-T segment, but the effects of injury in general have not been fully studied.

Effect of Certain Chemicals. Although various drugs and poisons known to act

⁵ The chart on page 25 of (12) shows that 0.02M NaHCO_3 in equilibrium with 5 per cent carbon dioxide at 25°C. and 37°C. will vary only between a pH of 7.30 and 7.40, respectively.

on the heart were tested in preliminary fashion, the effects produced by three are particularly suitable for presentation at this time.

Epinephrine has been employed to induce rhythmicity in heart muscle strips (3) apparently upon the assumption that it constitutes a perfectly physiological method. When enough has been employed to induce rhythmic activity (fig. 6) the S-T segment and T-wave are seen to become inverted, apparently the result of a relative anoxia. This phenomenon parallels the situation in intact animals (16) and is similar to the state produced by increased rate (*v.s.*). There is no reason to consider that such a change in the electrocardiogram is caused by coronary constriction as has been proposed (17).

The importance of the cardiac glycosides has led to much interest in their inti-

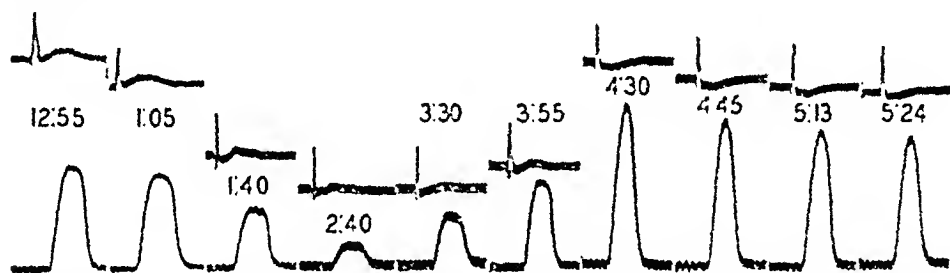


Fig. 7. EFFECT OF DIGITOXIN (1:10,000,000) added at 2:47 to a failing muscle. Note the depressed T-wave which develops in the electrogram (*upper tracing*) when the inotropic actions are fully developed (*lower tracing*).

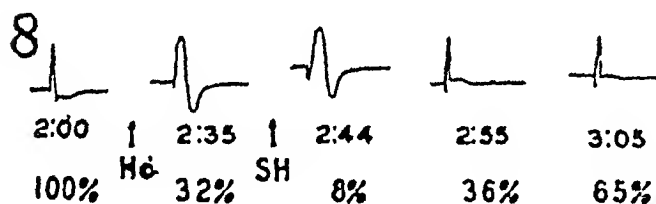


Fig. 8. EFFECT OF INHIBITION OF THIOL-containing systems of the papillary muscle produced by mercuric ion ($10^{-4}M$ $HgCl_2$ at *Hg*), and the reversal induced by thiosorbitol ($3 \times 10^{-3}M$ at *SH*). Contractile force is shown below as percentage of initial force.

mate cardiac actions. The inotropic action is again demonstrated in figure 7. In addition it is apparent that depression of the S-T segment, seen in clinical electrocardiograms, occurs also in the papillary muscle electrogram during the inotropic action of high concentrations of digitoxin.

A number of substances have been found to prolong the R-spike duration, notably potassium and quinidine, but the action of the mercuric ion is most informative. Figure 8 shows the prolongation of the R-spike and its fusion with the S-T segment. The ability of a thiol (the water-soluble, non-toxic thiosorbitol) to reverse completely this action of mercuric ion is clear-cut.

DISCUSSION

To decide whether the characteristic potential changes obtained from heart muscles can be ascribed to moving dipoles (2) or to membrane depolarization (7) or, indeed, to any specific cellular state (22) is not within the immediate province of this study, yet certain observations reported here bear indirectly upon this matter and directly upon the genesis of the electrocardiogram.

The conventional electrocardiogram (lead II, for example) normally consists of a series of deflections which are largely, if not entirely, negative with respect to the base line. The electrograms of small areas of isolated, uninjured, well-oxygenated and vigorously contracting mammalian heart muscle are precisely the same. It is evident that hypotheses which postulate interactions between the potentials produced by the two ventricles are not needed to explain the formation of the T-wave (18, 19) or the R-spike (20).

The sensitivity of the T-wave of these electrograms to anoxia induced directly by decreasing the available oxygen or indirectly by increasing the rate is equivalent to that of the limb lead electrocardiogram. This parallelism exists with respect to the actions of drugs; for example, inversion of the T-wave during the course of action of digitalis.

Inspection of most records reveals that regardless of the speed of the contractile process, as modified by changes of temperature and stimulation rate, the peak of the T-wave voltage change occurs at about the midpoint of contraction and T is invariably completed before relaxation is complete, suggesting that the T deflection may be intimately associated with the contracting state. However, this probably cannot be interpreted as constituting direct support for the hypothesis that the T deflection is of an electrokinetic origin (21) (e.g., streaming potentials developed during contraction) for it is not clear how such a phenomenon as the streaming potential could be affected by absolute or relative anoxia.

The form of the R-wave of the papillary electrogram is less responsive to conditions in the muscles' environment. Normally, it shows a single peak, brief in duration and wholly similar to its reflection in the limb leads. Decreased temperature prolongs the spike time in an orderly fashion ($Q_{10} = 2$) and it is probable that chemical reactions involving thiol groups are required for its rapid propagation.

An intriguing line of thought has been developed by Seifriz (22), viz. "Electrical forces in tissues are most often attributed to the unequal distribution of ions at membrane surfaces, but equally if not more probable as a source of electrical energy is the untold number of oxidation-reduction reactions which take place within a cell. Because we pick up some of the free energy by means of platinum electrodes and thus produce an electric current that can be measured, it is not implied that an electric current is produced in the system. We could just as well express the free energy in terms of a different scale . . . say, for instance, in terms of calories". Certain preliminary studies with metabolic substrates and inhibitors as well as the results with oxygen had already led to a rudimentary interpretation of some of the present results along this line. It appears to be a most fruitful idea.

The relevant literature contains many electrographic records which were apparently obtained from heart muscles in various degrees of depression as judged by the preparations described in this communication. Conclusions drawn from such experiments and mathematic expressions which describe such electrographic curves are not especially helpful. There is reason to believe that interpretations of electrograms of isolated heart muscle, whether obtained from a tissue immersed within a film or a much larger volume of electrolyte, can be made with more agreement when the tissue is known to be in a truly physiological state. The papillary muscle prepa-

ration should prove particularly helpful in this regard, provided cognizance is taken of the various experimental conditions shown here to affect the electrogram.

SUMMARY

Isolated papillary muscles from the right ventricle of the cat's heart have been found particularly suitable for examining the electrical and mechanical activity of mammalian myocardium.

The normal electrogram of actively contracting, well-oxygenated and uninjured mammalian myocardial cells in a thin film of electrolyte is totally monophasic. There is a fast electrically negative deflection ('R') followed by a slow deflection ('T') in the *same* direction, exactly as in the normal limb-lead electrocardiogram. Absolute anoxia (reduction in available O_2) or relative anoxia (increased rate of stimulation) both cause inversion of the T deflection in a manner predictable from clinical experience with the electrocardiogram. Temperature decrease ($42^\circ C.-17^\circ C.$) prolongs both the R and T deflection of the electrogram in an orderly fashion ($Q_{10} = 2$) and increases the amplitude and duration of contractions. Sympathomimetic amines, digitoxin and the mercuric (Hg^{++}) ion exert important effects on the electrogram and mechanogram.

There appears to be no further need for the previous complex explanations of the formation of the familiar limb-lead electrocardiogram which seems to be a peripheral reflection of the actual potentials generated by heart muscle cells.

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ACUTE EFFECTS UPON THE LUNGS OF DOGS OF LARGE INTRAVENOUS DOSES OF ALPHA-NAPHTHYL THIOUREA (ANTU)

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IN 1944 Dr. Curt P. Richter proposed to us that an examination of the effects of ANTU upon the production and flow of lung lymph be undertaken to attempt to learn more of the effects of this compound which proved so potent a cause of pulmonary edema. A few preliminary experiments upon dogs were done which showed that ANTU did cause an increase in the flow of lung lymph, coupled with pulmonary edema and transudation into the pleural sacs. At that time ANTU was upon the restricted list as regards publication, so that in 1945, when one of us (1) made use of a single experiment to illustrate the effects of the drug, he was not permitted to describe the compound other than as a 'thiourea derivative.'

In these first experiments, the ANTU, which is very insoluble in water, was ground finely and then suspended in a 6.0 per cent gum acacia solution to which sufficient sodium chloride had been added to give a concentration of 0.85 per cent. In a typical case a dog weighing 13.0 kgm. was anesthetized with nembutal and 26.0 cc. of the suspension were given intravenously. One cc. of the suspension contained 10 mgm. of ANTU powder, so that the dosage given was large—20 mgm. per kilogram. Increase in the flow of lung lymph began an hour after the injection was made and mounted rapidly over the next six hours. At autopsy this animal showed an extreme degree of pulmonary edema and had 130 cc. of fluid in the pleural sacs. The lung lymphatics were enormously dilated. The fluid they contained was almost red-cell free, and this, together with transudate collected from the trachea and that from the pleural sacs, contained practically identical amounts of protein. This pointed to a common source of the three fluids, the capillaries of the lungs. Microscopic examination revealed no kidney and no liver damage, nor effects of any sort in other organs. Richter had informed us that urine from rats killed by ANTU was fatal to other rats, but this evidence, that something toxic derived from the drug or the drug itself in very high dilution passed through the kidney, was not accompanied by renal changes obvious on microscopic examination. It is known (2) that foreign particles, in this case the suspended, finely divided ANTU crystals, are collected rapidly from the circulation by the liver. In this experiment, though sections of the liver revealed no abnormality, examination by polarized light showed many crystals arrested in or upon the Kupffer cells.

The animal under discussion breathed oxygen as soon as cannulation of the

right lymphatic duct was completed and practically constantly until the close of the experiment. After 2 hours and 20 minutes devoted to collection of lung lymph, both under natural breathing of oxygen and, to accelerate lymph flow, with artificial respiration by means of a pump which duplicated the rate and volume of natural breathing, ANTU was given. In summary, it was found:

1. One hour after ANTU injection there was definite evidence of increased production of lung lymph.

2. This increase mounted steadily for seven hours, when the animal was sacrificed by bleeding to death.

3. The protein content of the lung lymph first became greater, but during the last three hours of the experiment, when lymph flow increased very rapidly, the protein fell slowly.

4. During the last 2½ hours, minute volume of breathing rose from 5 to approximately 15 liters.

5. Blood pressure did not become dangerously low until practically the end of the experiment. Then, even though the animal was breathing oxygen, the arterial blood became exceedingly dark, indicating a high degree of terminal anoxia.

It was apparent from this and from similar experiments that ANTU caused acute lung edema in dogs, and that this condition was the dominant immediate effect of the drug. Furthermore, an increase in the production and flow of lung lymph was an early expression of ANTU action, and this effect could only be due to increase in the passage of water and solutes from the blood through the capillary walls. The compound was an agent which caused progressive, nonhemorrhagic and noninflammatory leakage from the lung capillaries, and since, by examining the production of lung lymph, objective evidence of the onset and effects of lung edema uncomplicated by cardiac breakdown could perhaps be secured, further experiments were done of which typical examples are now presented in this paper.

EXPERIMENTAL

Much experience with the effects of intravenous injection of ANTU suspensions, such as have been described in the introduction, brought out the uncertainty of this method of administration. The time between injection and the onset of lung changes, evidenced by lymph flow or respiratory changes, was extremely variable. Such prompt effects as have been described were obtained irregularly. Sometimes no change was observed during 6 to 12 hours, and then the animal passed rapidly into pulmonary edema. No explanation could be gained as to these capricious effects, which were so unpredictable as to make the use of ANTU for the study of the early physiological manifestations of lung edema impossibly time-consuming and troublesome.

Although Dieke and Richter (3) had shown quite wide variations in susceptibility of dogs to ANTU depending on age, it was felt that the insolubility of ANTU in water was clearly a major stumbling block. In 1946 DuBois, Holm and Doyle (4) reported experiments upon dogs and rats in which they admin-

istered ANTU dissolved in propylene glycol. They gave this solution intraperitoneally and found that in lethal dosage there was a prompt hyperglycemia followed by evidence that as poisoning progressed carbohydrate metabolism was profoundly altered. They noticed no immediate effects from intraperitoneal injection but concluded that hyperglycemia appeared so promptly as to make evident the rapid entrance of the intraperitoneal solution into the blood. The discovery of the solubility of ANTU in propylene glycol was at once utilized, the solution being given intravenously to dogs under barbiturate anesthesia. The injection must be given slowly, since breathing may fail if dosage is too rapid. Propylene glycol, in the amounts given, caused a fair degree of hemolysis. The protocols of two experiments follow:

APRIL 16, 1947. Dog 1, young adult, weight 12 kgm. Breathed air throughout experiment.

9:25 A.M. 8 cc. of 5 per cent nembutal, intravenously.

10:30. 100 cc. of Ringer's solution, intravenously.

11:00. 1 cc. of 5 per cent nembutal, intravenously.

11:30. 5 cc. of one per cent T-1824 in water intratracheally. This solution is absorbed practically entirely by lung lymphatics and causes the lung lymph to become blue, thus identifying the right lymphatic duct very clearly.

12:30 P.M. 1 cc. of 5 per cent nembutal, intravenously.

1:55. Arterial blood specimen 1, for blood gas analysis.

2:00. Finished cannulation of thoracic and right lymphatic ducts. Lung lymph from right duct dark blue. Thoracic duct lymph light blue, due in all probability to vascular absorption of dye given at 11:30 a.m.

2:15. 1 cc. of 5 per cent nembutal, intravenously.

2:23. Arterial blood pressure 168 mm. Hg.

2:24-2:32. Intravenous injection 12 cc. of 2 per cent ANTU in propylene glycol.

3:03. Arterial blood specimen 2, for blood gas analysis.

3:08. Arterial blood pressure 155 mm. Hg.

3:34. Rectal temperature 36.5°C.

3:54. Arterial blood specimen 3, for blood gas analysis; hematocrit, 54 per cent red cells; plasma protein, 5.96 grams per cent.

3:57. Arterial blood pressure 160 mm. Hg.

4:08. Arterial blood specimen 4, for blood gas analysis.

4:17. Arterial blood pressure 162 mm. Hg.

4:20. Rectal temperature 37°C.

5:12. Arterial blood specimen 5, for blood gas analysis; hematocrit, 62 per cent red cells; plasma protein, 6.31 grams per cent.

5:17. Arterial blood pressure 123 mm. Hg.

5:33. Arterial blood pressure 105 mm. Hg.

5:55. Arterial blood specimen 6, for blood gas analysis; hematocrit, 80 per cent red cells; plasma protein, 7.44 grams per cent.

5:56. Animal died.

6:00. Trachea tied. Autopsy performed, lungs being removed without wounding. No transudate in either pleural sac. The dye given intratracheally was mainly in the dorsal and dependent parts of the lower lobes. Tracheobronchial lymph nodes blue. Bluish lymphatics traced to node in upper right side of chest, from which efferents united in right lymphatic duct which was cannulated.

On section the cut surfaces of the lower lobes were wet, and many small and large bronchi exuded frothy bluish fluid, as did the trachea.

On microscopic examination, sections taken from the sternal and non-dependent part of the right upper lobe showed alveoli with thinned walls. These emphysematous areas were localized through the section. Here and there one found slight thickening of alveolar walls. There was no intra-alveolar transudate. Most striking and universally distributed through the section was wide dilatation of lymphatics about blood vessels and bronchioles. These were filled with light pink-staining fluid containing very few cells. There was no visible transudate in the lumina of the bronchioles.

Sections from dorsal parts of the lower lobes showed the same wide dilatation of perivascular and peribronchial lymphatics. Bronchioles frequently contained pink-stained nongranular material like that in the lymphatics, together with a few red and white cells. Here and there through the slides alveoli contained many red cells, and alveolar capillaries were frequently overfilled with red cells.

Figure 1 is a photomicrograph showing the changes we have described. In figure 2, the experiment is summarized. It is clear that a rise in the flow of lymph from the right duct, lung lymph, began less than an hour after administration of ANTU and continued unbrokenly until the end of the experiment. This fact means that lymph production, that is, transudation of fluid from the lung capillaries in excess of reabsorption, must have begun soon after ANTU injection and continued until the death of the animal. At autopsy, a moderate degree of pulmonary edema was evident on gross examination, and on microscopic search the finding was verified. But transudation into the alveoli was by no means general, and there was no fluid in the pleural sacs, a usual finding when ANTU acts over a longer period of time, as will be illustrated by the next experiment.

In our opinion, this experiment illustrates the early stages of pulmonary edema, when fluid leaving blood capillaries is in the main within the lung tissue and has not entered the alveolar air space to any large degree, though the fact that some alveolar filling was observed and that the trachea contained bloody froth indicated that the grosser, more widespread aspects of pulmonary edema were close at hand. Excess proteinized fluid, the transudate from lung capillaries, has ready access to the lymph capillaries of the lungs, and this fact is expressed by the increase in lymph flow and by the great dilatation of lymph channels observed microscopically (fig. 1).

Referring further to figure 2, line B, it is found that the rate of breathing began to rise shortly after increased lymph flow was established and grew steadily greater to the end of the experiment. Minute volume was also progressively enhanced. These changes in breathing cannot be related to anything dis-

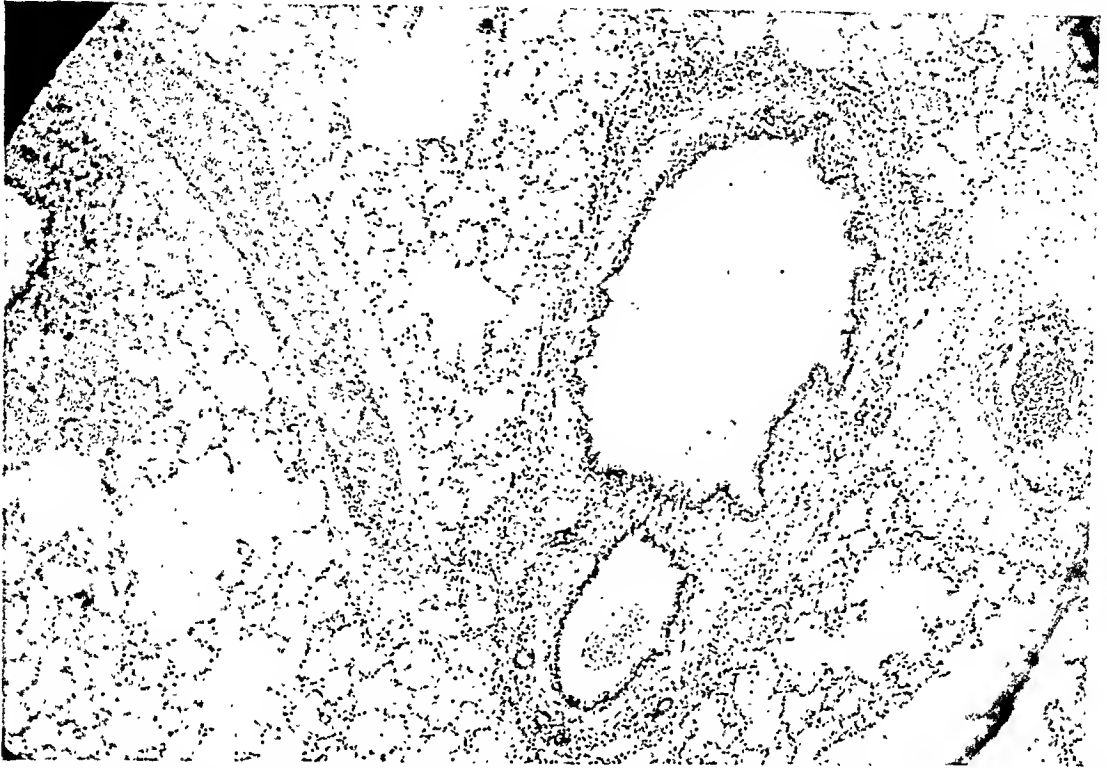


Fig. 1. LOW-POWER PHOTOMICROGRAPH of a section from the dorsal part of a lower lobe of the lung from *dog 1*. $\times 120$. Notice the greatly distended peribronchial and perivascular lymphatics and the negligible degree of alveolar transudation.

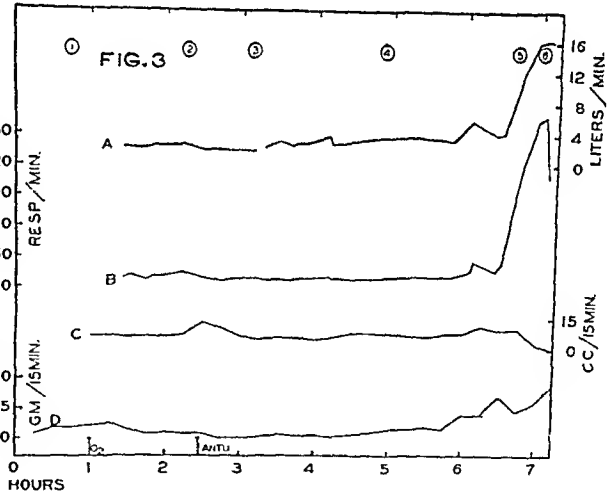
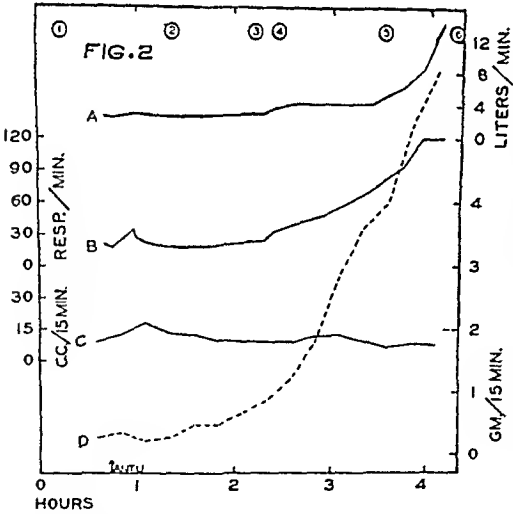


Fig. 2. SUMMARY OF THE COURSE of the experiment upon *dog 1*. *Line A*, minute volume of breathing in liters; *line B*, rate of breathing per minute; *line C*, flow of thoracic duct lymph in cubic centimeters per 15 minutes; *line D*, flow of right duct lymph in grams per 15 minutes. Circled numbers indicate times of securing arterial blood for gas analysis (table 1). *Abscissa*, time in hours; zero time 1:40 P.M. ANTU given at arrow.

Fig. 3. SUMMARY OF THE COURSE of experiment upon *dog 2*. *Line A*, minute volume of breathing in liters; *line B*, rate of breathing per minute; *line C*, flow of thoracic duct lymph in cubic centimeters per 15 minutes; *line D*, flow of right duct lymph in grams per 15 minutes. Circled numbers indicate times of taking arterial samples for blood gas analysis (table 2). *Abscissa*, time in hours; zero time 11:00 A.M. Oxygen administration begun at first arrow, ANTU injection at second arrow.

coverable in the blood by means of gas analyses. Table 1 gives the oxygen and carbon dioxide figures for the experiment, the specimens of arterial blood having been taken at the times indicated by the circled numbers in figure 2. Obviously there are no data in table 1 which explain the increase in breathing, both rate and minute volume. It is our opinion that the increase is neurogenic, and, lacking other lines of explanation, we are driven to the belief that transudation into the alveolar partitions, with abnormal distention and fixation of alveolar size, may cause stimulation of breathing through alveolar nerve endings; or ANTU, per se, which undoubtedly affects lung capillaries, may, at the same time, stimulate breathing. If this last were the case, it would seem that maximal stimulation of breathing should occur immediately following ANTU injection. The change in respiration is, however, something that develops gradually, ap-

TABLE 1. GAS ANALYSIS OF ARTERIAL BLOOD SPECIMENS FROM DOG 1

TIME	SPECIMEN NUMBER	OXYGEN CONTENT	OXYGEN CAPACITY	OXYGEN SATURATION	CARBON DIOXIDE CONTENT
<i>p.m.</i>		<i>vol. per cent</i>	<i>vol. per cent</i>	<i>per cent</i>	<i>vol. per cent</i>
1:55	1	23.57	25.55	92.2	40.27
2:24-2:32	Intravenous injection of ANTU				
3:03	2	23.17	25.91	89.4	38.05
3:54	3	23.62			38.62
4:08	4	23.91	25.70	93.0	37.58
5:12	5	25.93	29.64	87.5	32.0
5:56	Dog stopped breathing; specimen 6 practically at death				

pearing with the early phases of what will progress to extensive pulmonary edema if the animal survives long enough.

APRIL 28, 1947. Dog 2, young adult, weight 15.8 kgm. Breathed oxygen throughout experiment.

9:20 A.M. 11 cc. of 5 per cent nembutal, intravenously.

11:00. Finished cannulation of right lymphatic duct and started collection of lymph.

11:30. Finished cannulation of thoracic duct and started collection of lymph.

11:40. 150 cc. of Ringer's solution, intravenously.

11:45. Arterial blood specimen 1, for blood gas analysis.

1:05 P.M. Arterial blood pressure 176 mm. Hg.

1:17. Arterial blood specimen 2, for blood gas analysis; hematocrit, 40 per cent red cells.

1:18. Arterial blood pressure 172 mm. Hg.

1:19-1:25. Intravenous injection 15 cc. of 2 per cent ANTU in propylene glycol.

2:10. Arterial blood specimen 3, for blood gas analysis; hematocrit, 42 per cent red cells.

2:12. Arterial blood pressure 167 mm. Hg.

3:50. Arterial blood pressure 172 mm. Hg.

- 3:55. Arterial blood specimen 4, for blood gas analysis.
 4:50. Intravenous injection 50 cc. of Ringer's solution.
 5:20. 1 cc. of 5 per cent nembutal, intravenously.
 5:37. Arterial blood specimen 5, for blood gas analysis; hematocrit, 58 per cent red cells.
 5:40. Arterial blood pressure 152 mm. Hg.
 5:41. 1 cc. of 5 per cent nembutal, intravenously.
 6:00. Arterial blood specimen 6, for blood gas analysis; hematocrit, 79 per cent red cells.
 6:05. Arterial blood pressure 105 mm. Hg.
 6:15. Death of animal.
 6:25. Trachea tied and autopsy done at once. Lungs removed unwounded. Right pleural sac contained 25 cc. of straw-colored, slightly turbid fluid; protein content was 4.57 per cent. Left pleural sac contained 15 cc. of fluid of simi-

TABLE 2. GAS ANALYSIS OF ARTERIAL BLOOD SPECIMENS FROM DOG 2

TIME	SPECIMEN NUMBER	OXYGEN CONTENT	OXYGEN CAPACITY	OXYGEN SATURATION	CARBON DIOXIDE CONTENT
<i>a.m.</i>		<i>vol. per cent</i>	<i>vol. per cent</i>	<i>per cent</i>	<i>vol. per cent</i>
	Breathing air				
11:45	1	17.82	19.16	93.0	
12:00	Start breathing oxygen				
<i>p.m.</i>					
1:17	2	20.49	20.82	98.4	43.34
1:19-1:25	Intravenous injection of ANTU				
2:10	3	20.35	20.28	100.3	43.41
3:55	4	20.60	21.53	95.6	39.84
5:37	5	26.12	27.33	95.5	31.92
6:00	6	22.11	clotted		32.76
6:15	Dog dead				

lar appearance; protein content was 4.4 per cent. The last collection of lung lymph from the right lymphatic duct had a protein concentration of 4.54 per cent, indicating a common origin for pleural fluid and lymph.

The lungs, particularly upon the dorsal and dependent aspects, were livery and semi-solid. The appearances grossly were those of pulmonary edema and stasis.

On microscopic examination, sections of the right upper lobe from the sternal region, where gross appearances were more normal, showed overfilled blood vessels, widely dilated lymph trunks and no lung edema nor exudate in the bronchi. The alveolar walls in some regions contained capillaries overfilled with blood, in others, the thinned acellular alveolar partitions one associates with emphysema. Distended lymphatics were filled with light pink material emmeshing moderate numbers of leucocytes. Sections from the dorsal dependent part of the right lower lobe showed overfilled blood vessels, including capillaries in the alveolar walls. These walls were somewhat thickened, and the major number of alveoli appeared small as if compressed. Occasionally there were

small amounts of transudate in the alveoli, but none in the bronchi. Perivascular and peribronchial lymphatics were widely dilated and filled with pink-staining, fibrinous lymph, enmeshing occasional white cells. Edema of the visceral pleura is marked. Similar microscopic appearance of lungs from dogs and rats poisoned with ANTU are admirably described and illustrated in a recent paper by Latta (5).

Peribronchial lymph nodes were not abnormal.

In figure 3, the general course of *experiment 2* is given. This experiment was in agreement with *experiment 1*. Where differences occurred, they were in degree and not in basic import. For example, respiratory increase occurred considerably after lung lymph flow began to rise, and failure of breathing was not so abrupt as in the first case. The presence of pleural transudate indicated a more advanced stage of ANTU action, which in our experiments was not as easy to obtain if oxygen was not used, since the anesthetized and supine animals succumb to anoxia, due to tracheal and bronchial blockage from frothy transudate before more widespread blood capillary leakage expresses itself.

In table 2 are found the blood gas data for *experiment 2*. Again, there is agreement with the findings reported in table 1 for the first dog.

DISCUSSION

Two typical experiments out of a long series bring out the facts that ANTU dissolved in propylene glycol, and given intravenously to dogs under nembutal anesthesia, induces abnormal escape of water and plasma proteins from the pulmonary capillaries. If any degree of leakage from systemic vessels occurs simultaneously, it is so small as to escape notice.

This specific effect of ANTU results in increased lymph production and lymph flow. Since fluids containing the blood proteins are not absorbed by the lung capillaries except in traces (6), their removal from the lung parenchyma depends upon entrance into lung lymphatics with eventual delivery to the blood via the right lymphatic duct, or by entrance into alveoli with eventual lysis or outward transport along the air passages. Since the lung lymphatics, though quite voluminous (7), are restricted in their delivery of lymph by the small size of the right lymphatic duct, any increase in transudate from lung capillaries tends to cause pulmonary edema. In the case of ANTU, which acts so specifically upon these capillaries, the effects are rapid and progressive, so that poisoned animals soon drown in transudate. An increase in the flow of lung lymph constitutes the first functional expression of what will soon become extensive lung edema. Following the change in lymph flow, there is enhanced rate and minute volume of breathing, and this stimulation of breathing has not been shown to be due to changes in oxygen or carbon dioxide in the blood. It is not a central but a peripheral effect which accompanies distention of alveolar partitions by transudate.

No one can assign a final cause for the heightened breathing which accompanies rapid transudation of fluid from the lung capillaries. It is not a reaction favorable to the organism, and it may produce defective aeration of the blood.

Also, the very intensity of respiratory movement causes extensive frothing of the proteinized transudate in the air passages, and, in our opinion, the anoxia resulting from blockage of air movement by foam is the eventual cause of death. Inhalation of pure oxygen will delay death, but we know of no measures which will save ANTU-poisoned animals after increased lymph flow is definitely present, forecasting progressive lung edema.

SUMMARY

Dogs under nembutal anesthesia and given intravenously 2 per cent alpha-naphthyl thiourea (ANTU) in propylene glycol, one cc. per kilogram, developed fatal pulmonary edema. The first evidence of changes leading to edema was increase in the flow of lung lymph. Greater rate and minute volume of breathing followed. Blood gas analyses indicated that the hyperpnoea of rapidly developing pulmonary edema was peripheral in origin.

Detailed protocols are given for two typical experiments.

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EFFECT OF ANOXIC ANOXIA ON MYOGLOBIN CONCENTRATION IN STRIATED MUSCLE

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THE characteristics of myoglobin *in vitro* have been demonstrated to be those of a respiratory pigment, but the rôle of myoglobin *in vivo* has yet to be fully established. The literature to date affords two substantiated concepts with regard to its physiologic nature; namely, that myoglobin exists in striated muscle as a distinct heme pigment, and that it may act as a short-time oxygen store in the cell to tide the muscle over from one contraction to the next (1).

The study here reported was undertaken to determine whether or not myoglobin plays a part in the adaptation response of the organism to chronic hypoxia. Specifically, the study was designed to elucidate the physiologic effect of high altitude hypoxia on the myoglobin concentration in specific muscles of albino rats, and to disclose the extent, if any, of parallelism between changes in the myoglobin content of muscles and the increase in hemoglobin content of blood which occurs because of altitude hypoxia.

EXPERIMENTAL

Albino rats were subjected to a simulated altitude of 25,000 feet for four consecutive hours daily, from 12 to 312 days. The condition of the animals and their adaptation response to exposure were ascertained by periodic physical inspection, and by body weight, blood hemoglobin content and hematocrit determinations. The hematocrit values were obtained with Van Allen tubes. Hemoglobin concentrations were determined by hemolyzing one part of whole blood from the tail in 250 parts of distilled water, centrifuging, and measuring the densities of the resultant solution of oxyhemoglobin with a Beckman Spectrophotometer¹ at the wave lengths 5100, 5410, 5600 and 5770 Å. The value for hemoglobin recorded was the average concentration calculated from the four densities for the wave lengths above, using the specific extinction coefficients for oxyhemoglobin at those wave lengths determined by Horecker (2).

Except for the daily periods spent at altitude, the exposed animals and their designated controls were caged together and kept on the same diet, to which they had unrestricted access. After specific periods of exposure to simulated altitude, pairs of experimental animals and their controls were killed. The gastrocnemius and soleus muscles were dissected out and analyzed for their myoglobin and hemoglobin contents by methods of analysis (3, 4) reviewed here insofar as they pertain to the data presented.

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¹ A Beckman Spectrophotometer was used throughout this study. All measurements were made with a slit width of .04 mm.

The tissue sample was frozen in dry ice and hammered to a fine powder. A weighed amount of the powder was homogenized with a known volume of distilled water (3) in the ratio 1.2 to 2.4 gm. of sample per 10 ml. H_2O . The homogenate was centrifuged to remove the extracted meat residue, and the supernatant (pH 5.9–6.2) decanted and heated rapidly in a water bath to a temperature of 53–55°C. Within the limits of pH and temperature stated, proteins producing turbidity in the supernatant were coagulated and removed by centrifugation and filtration without significantly affecting the concentration of myoglobin in the extract (4).

The cleared extract was buffered with M/2 phosphate buffer pH 7.1–7.2 and divided into two portions.

a) The chromoproteins in one portion were oxidized by the addition of a few particles of powdered potassium ferricyanide, and converted to cyan-metmyoglobin and cyan-methemoglobin by the addition of a small crystal of KCN. The sum total of myoglobin and hemoglobin in the mixture was calculated from the density of this preparation at the wave-length 540 $m\mu$ by means of the formula, $c = \frac{D}{E \times L} \times d.f.$, where c is the total concentration of chromoproteins in moles or equivalents per liter, D is the optical density of the solution at 540 $m\mu$, E is Drabkin's molar extinction coefficient 11.3×10^3 for cyan-metmyoglobin at 540 $m\mu$ (5), L is the thickness of the absorbing layer, and $d.f.$ is the dilution factor due to buffering.

b) The chromoproteins in the second portion were reduced with dithionite in an atmosphere of carbon monoxide to convert the chromoproteins to a carbonyl-myoglobin and carbonyl-hemoglobin mixture;² and the absorption of the preparation in the visible range was studied for indications of chromoprotein denaturation and, in their absence, for the specific wave-length densities required to calculate the heme pigment fractions in solution (fig. 1).

The molarities for the individual hemoglobin and myoglobin components of the extract were calculated from the densities at the wave lengths 568 $m\mu$ and 538 $m\mu$, by means of the formulas,

$$[MbCO] = \frac{D_{568} \times E_{538}^{Hb} - D_{538} \times E_{568}^{Hb}}{E_{568}^{Mb} \times E_{538}^{Hb} - E_{538}^{Mb} \times E_{568}^{Hb}} \times d.f.,$$

$$\text{and} \quad [HbCO] = \frac{D_{568} \times E_{538}^{Mb} - D_{538} \times E_{568}^{Mb}}{E_{568}^{Hb} \times E_{538}^{Mb} - E_{538}^{Hb} \times E_{568}^{Mb}} \times d.f.,$$

where $[MbCO]$ and $[HbCO]$ are the molar concentrations of carbonylmyoglobin and carbonyl-hemoglobin; D_{568} and D_{538} are the measured densities of the solution containing MbCO and HbCO at wave lengths 568 and 538 $m\mu$, respectively; E_{568}^{Hb} and E_{538}^{Hb} , the molar extinction coefficients of 14.5×10^3 and 14.8×10^3 for HbCO at the wave lengths 568 and 538 $m\mu$, respectively; E_{568}^{Mb} and E_{538}^{Mb} , similarly the molar extinction coefficients of 11.8×10^3 and 14.8×10^3 for MbCO at the wave-lengths 568 and 538 $m\mu$, respectively (fig. 1); $d.f.$, the dilution factor for correcting dilution due to buffering, and the thickness of the absorption layer is 1.00 cm. (6).

In order to compare the myoglobin or hemoglobin determinations on different samples, the calculated molar concentrations were converted to milligrams of Mb or milligrams of Hb per gram of muscle by means of the formula,

$$\begin{array}{c} Mb \text{ mg/gm. muscle} \\ \text{or} \\ Hb \text{ mg/gm. muscle} \end{array} = \frac{17,000 \times [MbCO] \text{ or } [HbCO] \times w.f.}{\text{gm. muscle sample}},$$

where 17,000 is the equivalent weight assumed for Mb and Hb, and $w.f.$ is the fraction of a liter equal to the sum of water used to transfer the powdered tissue sample to the homogenizing tube plus 75 per cent of the sample weight, assuming that 75 per cent is the water content of the muscle sample (7).

Identical processing was employed for each pair of experimental and control animals in the series to make their results directly comparable for the effect of altitude

² The quantity of cytochrome in rat gastrocnemius and soleus muscle extracted by this process is negligible.

exposure. To make the findings for all pairs directly comparable, the concentrations of myoglobin for each exposed animal and for its specific control are expressed in the form of a whole number ratio (table 2, col. 6). By thus equating all variables but the duration of altitude exposure for the different pairs, each ratio expresses a decreased or increased myoglobin concentration for the exposure time given in column 4, depending upon whether the ratio is less or more than 1.00. Comparison of the

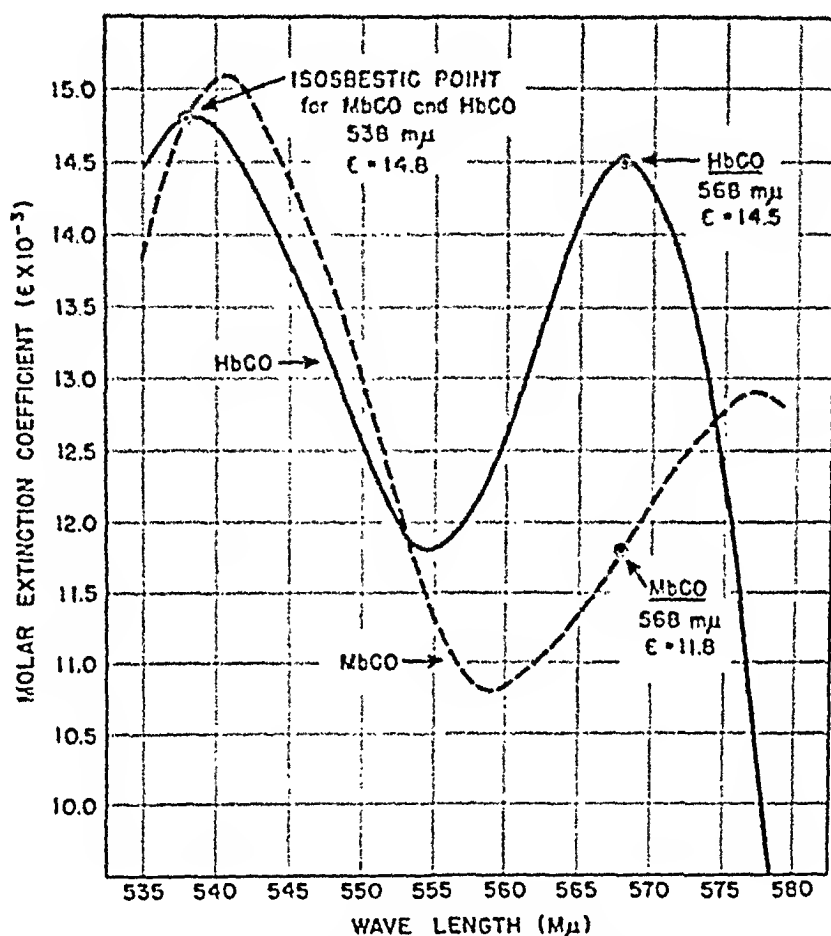


Fig. 1. ABSORPTION CURVES for carbonyl-myoglobin (MbCO) and carbonyl-hemoglobin (HbCO) for the wave-length span 536-578 mμ. Determinations of the carbonyl-myoglobin and carbonyl-hemoglobin concentrations for mixtures of both heme pigments were based on the optical densities of the mixture and the respective molar extinction coefficients of the pigments at the wave length 538 and 568.

ratios for two or more pairs of animals gives an index to the relative changes in myoglobin concentration due to the differences in time spent at altitude.

With suitable modifications of the method reviewed above, analyses were made also of the hearts of animals numbered 6 to 12 in this study. Refinement of the modified method for rat heart analysis was carried to a point where significant but only roughly quantitative results were obtained.³ These preliminary data appear to

³ Modifications in the method described were required to make it applicable for the analysis of heart muscle. a) Heat coagulation of the interfering proteins in a water extract of cardiac muscle does not take place within the temperature range given above for skeletal muscle extracts. This is due in part to the greater alkalinity of the heart extract (pH 6.75-6.90) as compared with that for the

corroborate the observation reported for heart tissue by Hurtado *et al.* (8) that the concentration of myoglobin in cardiac muscle is increased by high altitude hypoxia.

Despite the significance of a study of myoglobin in heart muscle, this was not the primary purpose of the investigation. As a consequence, and because of apparent corroboration by the preliminary findings of observations already published, further attempts at methodologic refinement for more precise quantitative estimations of cardiac myoglobin were postponed. The observations made pertaining to cardiac myoglobin concentration and the implications drawn from these observations are

TABLE 1. HEMOGLOBIN, HEMATOCRIT AND BODY WEIGHT CHANGES IN A TYPICAL EXPERIMENTAL MALE RAT¹ AND CONTROL¹ SHOWING EFFECT OF ALTITUDE EXPOSURE

DATE	BODY WT., GM		HEMATOCRIT TOTAL CELL VOL. % ³		HEMATOCRIT RED CELL VOL. %		HEMOGLOBIN CONC., GM/100 ML. BLOOD	
	Exp.	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.	Contr.
Nov. 4 ²	211	190	48.8	45.6	47.2	44.2	14.2	13.6
Nov. 7	208	206	49.9	47.3	49.0	46.1	14.7	13.7
Nov. 21	215	222	69.3	49.0	68.3	48.0	20.4	15.5
Dec. 1	218	240	70.5	49.8	70.0	48.2	21.1	14.0
Dec. 15	216	264	77.1	50.7	76.7	49.1	22.7	15.4
Dec. 29	232	311	78.2	51.1	77.8	50.0	23.8	15.3
Jan. 5	241	332	79.3	49.0	78.5	48.0	23.5	14.6
April 7	282	340	80.4		80.0		23.9	

¹ These animals are listed as 6X (Experimental) and 6C (Control) in table 2.

² One day prior to initiation of altitude exposure of experimental animal. The animal was exposed to a simulated altitude of 18,000 ft. for the first 2 days, and to 25,000 ft. thereafter.

³ Total cell volume includes the buffy layer above the packed red cells.

mentioned in the following sections, but no pertinent quantitative data are given in this report.

RESULTS

Body Weight, Hemoglobin and Red Cell Volume Changes

These changes in the albino rat following altitude exposure have been dealt with intensively by other investigators (9-12). The uniformity of these changes in the exposed animals was such that the data for a single pair, given in table 1, may be regarded as typical.⁴ Increases in the red cell hematocrit values and hemoglobin concentrations began soon after exposure to altitude was initiated, and continued for approximately 6 to 10 weeks during the course of exposure before showing indications of having reached a steady state. There were no exceptions to the trend of these changes in any of the exposed animals.

gastrocnemius and soleus muscle (pH 5.9-6.2). *b*) The absorption spectra for cardiac muscle extracts differ from those of the gastrocnemius and soleus to an extent requiring correction for chromoprotein absorption other than that due to myoglobin and hemoglobin.

⁴ These animals are listed as 6X (exposed) and 6C (control) in table 2.

Hemoglobin Content of Muscle Samples

The concentration of blood hemoglobin in the tissue extracts of animals exposed to altitude increased progressively with the duration of altitude exposure and paral-

TABLE 2. EFFECT OF ALTITUDE HYPOXIA ON MYOGLOBIN CONCENTRATION IN THE GASTROCNEMIUS AND SOLEUS MUSCLES OF ALBINO RATS

RAT	SEX	TERMINAL AGE, DAYS	DAYS OF EXPOSURE TO 25,000 FT. ALT., 4 HRS/DAY	CONC. OF MYOGLOBIN (GASTR. AND SOLEUS)	
				Mg./gm. of sample	Ratio of Mb. Exp/ Contr. ¹
1X ¹	M	200	14	2.00	1.19
1C ¹	M	200	0	1.68	
2X	M	210	23	1.82	0.81
2C	M	210	0	2.25	
3X	M	220	43	1.97 ²	0.95
3C	M	220	0	2.08 ²	
4X	M	230	47	2.37 ²	1.16
4C	M	230	0	2.04 ²	
5X	M	166	12	1.86	0.91
5C	M	165	0	2.05	
6X	M	300	152	1.57	0.66
6C	M	177	0	2.37	
7X	M	300	157	1.66	0.77
7C	M	310	0	2.16	
7X	M	310	163	1.46	0.70
8C	M	310	0	2.09	
9X	M	311	297	1.73	0.64
9C	M	310	0	2.72	
10X	F	200	186	1.34	0.76
10C	F	205	0	1.76	
11X	F	206	192	1.52	0.87
11C	F	205	0	1.75	
12X	F	326	312	1.56	0.86
12C	F	300	0	1.82	
13X	F	212	198	1.16 ²	

Miscellaneous Analyses

A	M	360	0	2.56	
B	M	360+	0	1.95	
C	F	250	0	1.93	
D ³	F	330	0	1.58	

¹ 'X' and 'Exp.'—Exposed animal. 'C' and Contr.—Control animal. ² M. Gastr. only.

³ Early pregnancy.

leled the rise in red cell volume closely. The hemoglobin concentrations for the animals 1X, 2X, 3X and 4X, exposed from 14 to 47 days, were 0.25, 0.41, 0.65, and 0.44 mg./gm. of muscle sample respectively. The hemoglobin values for the muscle samples of animals that reached a steady state, i.e. the group in which the exposure

period was 152 days or longer, ranged from 0.57 to 0.68 mg/gm. of muscle. The range of values for their controls was 0.08 to 0.20 mg/gm.

Although absolute values are used to express the concentration of 'tissue-trapped hemoglobin', these values should be considered only as roughly quantitative in significance. They do demonstrate that, all other conditions being the same, the animal with polycythemia had an increased amount of red cells trapped in its muscle sample at death as compared with the control; the amount of hemoglobin present having a positive correlation with the duration of altitude exposure and red cell volume increase.

Changes in Myoglobin Concentration

1. *Gastrocnemius and soleus muscles* (table 2, cols. 5 and 6). The analyses of the animals with 152 days or more of altitude exposure all show marked diminution of myoglobin concentration in the gastrocnemius and soleus muscles, regardless of the sex of the animal or the age at which exposure was initiated.⁵ No significant change in myoglobin concentration could be demonstrated for the group with an exposure period of from 12 to 47 days however.

It is interesting to note that of all the analyses made, the lowest myoglobin concentration in leg muscle was obtained on analysis of *rat 13X* (table 2). This one animal died during an exposure period at 25,000 ft. in the altitude chamber, after 198 days of previous exposure.

2. *Cardiac muscle*. The hearts of all exposed animals were grossly hypertrophied as evidenced by their size and weight. This condition due to altitude hypoxia has been previously described (9-12).

Heart analyses for changes in myoglobin concentration were made only for the animals numbered 6 to 12, of the group with 152 or more days of altitude exposure. In every instance the concentration of myoglobin per gram of heart muscle was significantly higher in the exposed animal than it was in the control. The findings therefore indicate both an increased concentration of myoglobin per gram of heart muscle, as well as an increase in the total content of cardiac myoglobin for the exposed animal.

DISCUSSION

The contrasting points of significance enumerated above may be summarized as follows:

1. There is no demonstrable change in concentration of myoglobin in the gastrocnemius and soleus muscles of rats exposed to altitude anoxia for periods up to 47 days, under the experimental conditions described. However, marked increases in blood hemoglobin concentration and circulating red-cell volume do occur during those periods.

2. There is a marked decrease in myoglobin concentration in the gastrocnemius and soleus muscles of rats exposed for 152 days or longer. However, the blood hemoglobin concentration and red-cell volume, after continuously increasing during the first 6 to 10 weeks of exposure, remain fairly stable thereafter and show little sig-

⁵ The age at which altitude exposure was initiated is the difference between the total days of exposure for the animal and its terminal age; ref. cols. 3 and 4, table 2.

nificant variation during the period of exposure in which a reduction in concentration of leg-muscle myoglobin is observed.

3. Heart tissue analyses indicate an increase both in content and concentration of cardiac myoglobin in the rats exposed to altitude anoxia. Analyses of skeletal muscle, on the other hand, show a markedly decreased myoglobin content following prolonged exposure.

Exposure to anoxia was made in a manner that called forth no increased activity from the gastrocnemius and soleus muscles of the rats at altitude. In fact, the possibility is present that activity of those muscles may have been somewhat inhibited by chronic intermittent anoxemia. It has even been reported that skeletal muscles under anoxic conditions have a greatly lowered capacity for energy production; in other words, they are capable of much less work at altitude than at sea level (13a).

Cardiac activity, on the other hand, is greatly augmented by exposure to anoxia; at first by a physiologic demand for an increased heart rate and cardiac output and, subsequently, by the added burden of an increased blood viscosity which is only partially compensated for by changes in the vascular system. The high red cell volume and hemoglobin content of the typical example in table 1 illustrate this hemodynamic burden imposed on the heart by prolonged altitude exposure. The cardiac hypertrophy observed in all exposed animals demonstrates a phase of the adaptation response to the increased activity imposed by these factors on the heart. Since myoglobin content of striated muscle increases with muscular activity (14a, 15), contrasting variations of myoglobin content in skeletal and cardiac muscle following altitude exposure, as well as the findings noted in 1, 2, and 3 above, suggest: *a*) that changes of myoglobin concentration in a specific muscle arising after exposure of an animal to anoxia depend on the change in activity of that muscle due to the effect of the anoxic environment on the animal; and *b*) that myoglobin concentration is not altered by the mechanism causing hemoglobin variations, nor do observed changes in myoglobin concentration appear to be direct anoxic adaptation responses.

An investigation by Whipple (14b) demonstrated that there is no direct parallelism between the hemoglobin and myoglobin concentration in dogs following severe anemias experimentally induced and prolonged by repeated bleedings. The present investigation indicates that the lack of parallelism between changes in concentrations of hemoglobin and myoglobin extends also into the range of polycythemia due to altitude exposure.

Hurtado and his associates, on the other hand, report that parallelism does exist between the myoglobin level and the hemoglobin level in polycythemia of altitude observed in dogs. They suggest that a myoglobin increase in such cases represents an important mechanism of adaptation to chronic anoxemia at the tissue level (8).

Their observations, made on dogs native to altitudes of 12,300 and 14,890 feet, cannot be compared directly with those herein reported, because of the obvious differences in the method of exposure to altitude, the altitudes at which exposure was made, the difference in species of animal used etc. Dill has occasion to mention the adaptability and phenomenal capacity for activity of dogs at high altitude (13b). The differences in our findings thus may be due in part to the different capacities

for activity at altitude of the animals employed. Nevertheless, the interpretation of Hurtado *et al.* that an increased concentration of myoglobin in dogs at altitude may represent a mechanism of adaptation to a condition of chronic anoxia, is one that cannot be reconciled with the findings here reported.

SUMMARY

A study was made to determine whether or not myoglobin plays a part in the adaptation response of an organism to chronic anoxemia. Quantitative estimations of the myoglobin in specific muscles were made on a series of albino rats exposed regularly but intermittently to a simulated altitude of 25,000 feet, for 12 to 312 days. The method of analysis employed is one which permits quantitative determinations of myoglobin to be made on extracts of unperfused muscle containing hemoglobin.

The data obtained indicates no direct relation between changes in myoglobin concentration and adaptation to a condition of altitude hypoxia. Skeletal muscle, in which activity is not enhanced by anoxia, eventually showed a decreased myoglobin content following prolonged altitude exposure; whereas cardiac muscle, in which activity is enhanced by anoxia, apparently showed an increased myoglobin content. These changes tend to indicate that the myoglobin content of a muscle is determined by muscular activity rather than by anoxia, even in an anoxic environment.

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EFFICIENCY OF VARIOUS TYPES OF ARTIFICIAL RESPIRATION AT HIGH ALTITUDES¹

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IN SPITE of the importance of the correct technic for the administration of resuscitation, few experiments have been reported which evaluate the efficiency of the various methods for artificial respiration at normal atmospheric pressures; no studies have been reported at high altitudes.

The studies reported have been a comparison of only two or three technics and many of these have been carried out on human subjects who had voluntarily suspended respiration. It has been shown by Cordier (1) that this is probably an unsatisfactory experimental state since, when the Schafer method was used on subjects voluntarily suspending respiration, the pulmonary ventilation was 360 to 1000 cc. per cycle while nonrigid cadavers showed an exchange of 20 to 50 cc. per cycle. Comroe and Dripps (2) reported results using the Schafer and Eve methods on two patients with respiratory arrest caused by physical disability which showed that the Schafer method gives less pulmonary ventilation than the Eve method; however, they do not feel the Eve method presents the best possible technic for artificial respiration. Steinberg and Dietz (3) compared the efficiency of manual and mechanical methods of resuscitation on dogs after respiratory arrest produced by anoxia and concluded that the pulmotor type of artificial respiration was superior. In a survey of various life-saving organizations giving resuscitation to human beings, Ross (4) showed that the mechanical resuscitators were used more than manual methods without evidence of injury to the patients. Recently Swann *et al.* (5) reported results on resuscitation in which they studied the terminal physiological events following various types of fatal accidents in an attempt to better analyze resuscitation procedures. They concluded that the systolic arterial pressure gave a good index of the imminence of death and using this found that insufflation of the lungs gave a slight margin of superiority over manual methods for artificial respiration.

In a previous study (6) we investigated a number of respiratory and circulatory functions in the rat at low barometric pressure and were impressed with the apparent differences in respiratory control and performance. This suggested the desirability of an investigation of the various methods of artificial respiration using the rat as the experimental animal. Five types of artificial respiration were evaluated first at Denver's altitude (bar. press. 630 mm.) and then at altitudes approximating 40,000 feet (bar. press. 140 mm.). The types studied were: Eve tilt board; Schafer prone pressure; Pulmotor or 'suck and blow' type; Drinker respirator; and Thunberg barospirometer. Also, the effectiveness of diffusion respiration and of a number of respiratory stimulants was studied at normal atmospheric pressure. Pulmonary ventilation, the resumption of respiration and restoration and maintenance of circulation were used to evaluate the effectiveness of these types of artificial respiration.

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EXPERIMENTAL

Adult white rats of the University of Denver strain weighing 250 to 350 grams were used and thus they had whatever advantage may be derived from being raised at an altitude of 5280 feet. Sodium pentobarbital in a dose of 50 mg/kg. was used as an anesthetic. Continuous blood pressure records were made on all animals by the method described in an earlier paper (6). In all animals the trachea and carotid artery were cannulated for administration of artificial respiration and to determine blood pressure. Respiratory stimulants were administered by cannulation of the jugular vein. At Denver's elevation cessation of respiration was brought about by curarization or by allowing the animal to breathe pure nitrogen. At high altitudes apnea occurred due to the low oxygen tension in the air the animal was breathing. Simulated high altitudes were produced by reducing the pressure in a decompression chamber at a rate equivalent to 2000 feet per minute. The circulation of air through the chamber was 4 liters of room air per minute at 40,000 feet. Artificial respiration was not given until an apnea occurred accompanied by a fall in blood pressure to about 50 mm. which was considerably below the 'point of failure' in the dog of 75 mm. reported by Swann. Artificial respiration was given at a constant rate of 35 cycles per minute for at least 5 minutes in all methods.

Eve Tilt Board, Denver Altitude. The animal was fastened to a metal tray which could be rocked through an angle of 35° at a constant rate of 35 cycles per minute. The trachea of the animal was cannulated to insure free passage of air to the lungs and to make it possible to measure pulmonary ventilation. A total of 10 animals was used; in 4, respiratory arrest was produced by intravenous injection of Intocostrin (2 mg/kg.) and in 6, apnea was brought about by allowing the animals to breathe pure nitrogen. When a rat breathed pure nitrogen apnea occurred within a minute accompanied by a precipitous fall in blood pressure. After one to two minutes of apnea a slow powerful heart was noted which resulted in a return of the blood pressure to normal or above and a spontaneous recovery of respiration. If the animal was allowed to breathe air at this time there was full recovery; however, if nitrogen administration was continued a second apnea, accompanied by a fall in blood pressure, occurred in 6 to 8 minutes. This period varied from 2 to 20 minutes. If no artificial respiration was given at this point, the animal did not recover. This second apnea was used in all experiments where respiratory arrest was induced by nitrogen inhalation. None of the 10 animals studied could be resuscitated by the Eve tilt board method. During the administration of the artificial respiration, sensitive one-way valves were attached to the tracheal cannula but no pulmonary ventilation could be demonstrated. Neither respiratory movements nor maintenance of blood pressure could be observed in any of these animals.

Schafer Prone Pressure Method, Denver Altitude. The apparatus used to give artificial respiration to the rat by this method consisted of a board upon which the rat was placed stomach down and a soft rubber tube about one inch in diameter supported on the outside by a metal band which was fastened over the back of the rat. The tube was alternately inflated under a pressure of 100 mm. Hg and then deflated to room pressure. This applied pressure periodically to the lower portion of the rat's back and sides in a manner simulating the Schafer method on human beings. Ten

animals were studied; in 5, respiratory paralysis was produced with Intocostarin and in the others nitrogen was administered until the second apnea developed. None of the rats showed a circulatory response nor was it possible to demonstrate any pulmonary ventilation during the administration of artificial respiration.

Pulmotor, Denver Altitude. The pressures used for artificial respiration by this method were 15 to 20 mm. positive pressure and 0 to 10 mm. negative pressure. This produced a pulmonary ventilation of 175 to 225 cc. per minute. Three rats were given Intocostarin intravenously and 9 were forced to breathe pure nitrogen. Two of the curarized rats maintained adequate circulatory function during the period of respiratory paralysis (about 20 minutes) and when artificial respiration was stopped, they resumed normal respiration. Of the 9 rats receiving nitrogen inhalation, 6 showed full recovery of respiratory and circulatory function and one showed a circulatory response but no respiratory response.

Drinker Respirator, Denver Altitude. The respiratory chamber consisted of a liter jar fitted with an air-tight lid. The rat was placed on a tray fastened to the lid and the trachea attached to a tube sealed in the lid so that the lungs were open to the atmosphere outside the chamber. Alternately, positive and negative pressures of 20 to 25 mm. Hg were introduced into the chamber. Respiratory paralysis was induced in 5 rats with Intocostarin and apnea produced in 7 animals by nitrogen inhalation. All 5 of the animals receiving Intocostarin maintained a satisfactory blood pressure during the period of artificial respiration (20 minutes), but only 4 showed a respiratory response. Four of the rats on nitrogen recovered both respiratory and circulatory functions, the other 3 showed neither response. The pulmonary ventilation produced by this method was from 75 to 125 cc. per minute.

Thunberg Barospirator, Denver Altitude. The barospirator is an apparatus for giving artificial respiration by means of variations in the pressure in a closed chamber containing the animal. Primarily the animal derives the pulmonary ventilation by the compression and expansion of the air normally remaining in the lungs; however, due to the resistance of the trachea and bronchi some movement of the chest also occurs. The rat was placed in a closed jar and air under a pressure of 250 mm. Hg was forced in and allowed to escape at the rate of 35 times per minute. The application of this pressure gave a rather unique blood pressure recording since the tambour remained at normal pressure while the recorded blood pressure of necessity rose and fell with the change of pressure in the jar. Respiratory paralysis was produced in 4 rats with Intocostarin; 2 of the animals recovered both circulatory and respiratory functions in about 30 minutes and the third animal maintained an adequate blood pressure for one hour and fifty minutes, but respiration could not be restored. Cessation of respiration was produced with nitrogen in 8 animals and of these, 4 were completely resuscitated with the barospirator.

Diffusion Respiration. It has been known for many years that life can be maintained in apneic animals for a long period of time without external respiration if a stream of oxygen is directed down the trachea (7). One problem that arises in this type of respiration is the effective removal of carbon dioxide since carbon dioxide has a greater molecular weight than oxygen and thus diffuses more slowly. Helium, because of its low molecular weight and high diffusability, has been used clinically

for a number of years in the treatment of respiratory diseases such as asthma. In view of this it was thought that helium might facilitate the removal of carbon dioxide if a helium-oxygen mixture were used in diffusion respiration. Rats were allowed to breathe the diffusion mixture for 5 to 6 minutes in order to displace the air from the lungs, then respiration was stopped by intravenous injection of Intocostrin and diffusion respiration started. The length of time the animal was able to maintain its blood pressure was used to evaluate the method. Seven animals were diffused with 100 per cent oxygen with an average survival time of 26 minutes; 5 animals diffused

TABLE 1

DRUG	DOSE	NUMBER OF ANIMALS USED	RESPONSE, NO. OF ANIMALS	PER CENT RECOVERY
	mg./kgm.			
Caffeine.....	100	14	1	7
Coramine.....	75-125	6	6	100
Strychnine.....	1	7	5	71
Picrotoxin.....	1	9	4	45
Metrazol.....	10	6	3	50
Potassium cyanide.....	0.1 cc. of .05% soln.	8	4	50

with a 20-80 helium-oxygen mixture showed an average survival time of 14 minutes, and 5 animals diffused with a 50-50 helium-oxygen mixture showed an average survival time of 15 minutes. No beneficial effect could be demonstrated by the addition of helium to the diffusion mixture, but rather there was a tendency to decrease the effectiveness of the method probably due to a decrease in the oxygen tension in the lungs because of the dilution with helium. It is interesting to note that a rat breathing only air before curarization can maintain a satisfactory blood pressure for 4 to 5 minutes without any form of artificial respiration.

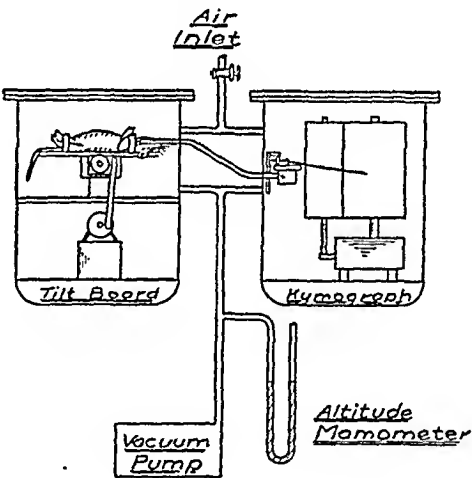


Fig. 1

Respiratory Stimulants. Six drugs were administered intravenously to determine their effectiveness in restoring breathing following respiratory arrest produced by nitrogen inhalation. The results are given in table 1.

The one animal that showed a response with caffeine responded on four successive trials showing complete respiratory recovery after each trial. The effectiveness of Coramine is interesting in view of the work reported by Flickinger and Adler (8) in which they concluded that Coramine was not effective in dogs and in fact when given in the above dose may have contributed to the death of some of the animals. Strychnine, though nearly as effective as Coramine, had to be given in a dose close to the convulsive level which resulted in the production of convulsions in most of the animals at about the same time that respiration was resumed.

Ere Tilt Board, High Altitude. This method was easily adapted for giving artificial respiration at high altitude as is shown in figure 1. The pressure in the chamber was reduced until there was a cessation of respiration accompanied by a fall in blood pressure. This occurred at an elevation of about 40,000 feet. Artificial respiration was then given for 5 to 10 minutes by the same procedure as at low altitude. Nine rats were used but none of these showed any respiratory or circulatory response.

Schafer Prone Pressure Method, High Altitude. The apparatus used for simulating high altitude and giving artificial respiration by this method is illustrated in figure 2. The pressure tank was maintained at about normal atmospheric pressure and when it was necessary to give artificial respiration, stopcock 2 was closed and

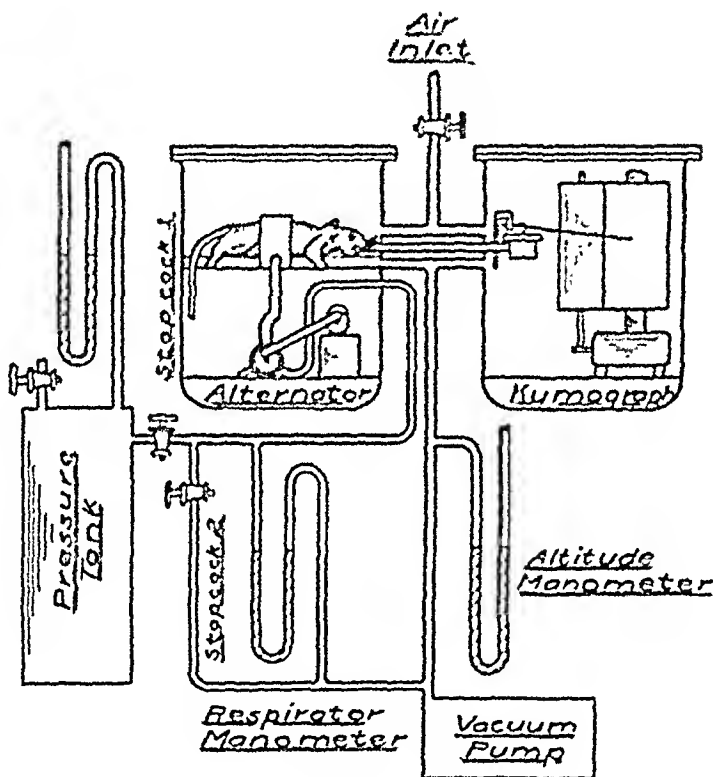


Fig. 2

stopcock 1 opened until a pressure of 75 to 90 mm. was obtained as shown on the respirator manometer. This resulted in applying the pressure to the Schafer apparatus in the chamber in the same manner as at low altitudes. Fifteen rats were taken to an altitude of about 40,000 feet and maintained until a definite apnea developed at which time artificial respiration was applied. Circulatory and respiratory responses were produced in 6 of the 15 animals.

Pulmotor, High Altitude. Because of the manipulative difficulties ever present at high altitudes, artificial respiration with the pulmotor was accomplished by applying pressure but no vacuum; Henderson and Turner (9) have stated that this technic represents a very efficient method for resuscitation. The apparatus used is diagramed in figure 3. The gas (air or oxygen-carbon dioxide mixture) contained in the oxygen spirometer was maintained at normal atmospheric pressure and was in-

roduced into the alternator at a pressure of 15 to 20 mm. Hg greater than that in the decompression chamber. This was accomplished, as with the Schafer apparatus, by closing stopcock 2 and properly adjusting stopcock 1. A pulmonary ventilation of 5 to 8 cc. per cycle was produced under these conditions. Two series of experiments were carried out, one in which artificial respiration was given with air and the other in which it was given with a 95 per cent oxygen-5 per cent carbon dioxide mixture. In series one, 11 animals were used; 5 of the animals showed both a circulatory response and respiratory response, and one showed only a circulatory response. Nineteen animals were used in the second series; 10 were resuscitated as shown by circulatory and respiratory recovery and 2 showed only vaso-motor response. In

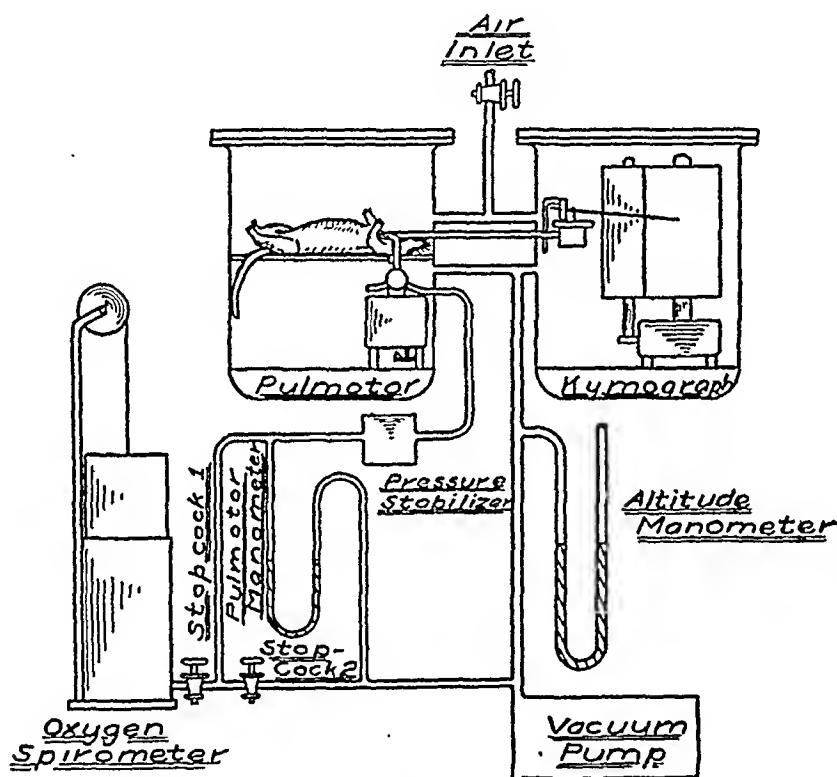


Fig. 3

all of the animals that were resuscitated it was observed that the heart and circulation responded first, in some cases several minutes before respiration was resumed.

Drinker Respirator, High Altitude. The Drinker respirator as adapted for use at high altitude is shown in figure 4. The spirometer was connected to a metal cap which fit loosely over the end of the tube from the trachea so that artificial respiration could be given with gas mixtures of any desired composition. The vacuum tank was maintained at a pressure less than the decompression chamber at all times during the ascent so that artificial respiration could be given whenever necessary. When artificial respiration was given, stopcock 2 was closed and a negative pressure of 55 to 65 mm. Hg developed by the proper adjustment of stopcock 1. This pressure difference between the respiratory and decompression chambers produced a pulmonary ventilation of 3 to 5 cc. per cycle, (100 to 175 cc/min.). In the first group,

resuscitation was attempted on 9 rats and none showed either respiratory or circulatory response. In the second group, 3 rats were studied by the same procedure except an oxygen-carbon dioxide mixture was given during the period of artificial respiration, but again none of these animals was resuscitated. It has been shown previously that one method by which a rat adjusts to severe anoxia is by decreasing his body temperature (6). If the environmental temperature were too high, it would not be possible for the animal to make this adjustment. At the time these experiments were run the laboratory temperature was high and the chamber containing the animals often reached a temperature of 35°C . It was concluded that this might account for the difficulty encountered in resuscitating these animals. In the next two groups

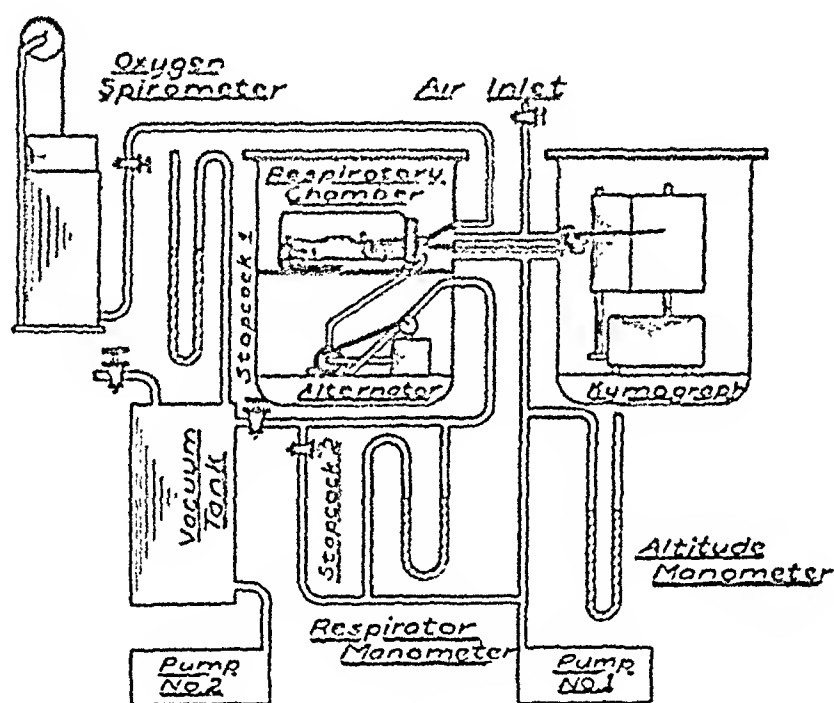


Fig. 4

of experiments the chamber temperature was reduced by placing a metal container containing ice around the chamber; a temperature of 15 to 20°C . could be maintained by this method. Eleven animals were studied at this lower environmental temperature without extra oxygen administration and 5 regained both respiratory and circulatory function. A record of the blood pressure changes during the response is shown in figure 5. Of 15 animals tested at the lower temperature and receiving the oxygen-carbon dioxide mixture during resuscitation, 8 showed complete response and 3 showed only circulatory response. This demonstrates very vividly the importance of the environmental temperature as it pertains to anoxia in the rat.

Barospirator, High Altitude. Artificial respiration at high altitude by this method was given with the apparatus illustrated in figure 6. Increased oxygen concentrations could be given during the resuscitation by maintaining a high oxygen atmosphere around the end of the tracheal cannula. This was accomplished by means of a small cap fastened inside the chamber over the end of the tracheal cannula

and attached to a tube from the spirometer. A pressure of 100 to 120 mm. was introduced into the chamber from the pressure tank by the same adjustment of the stop-cocks as in the other methods. In all experiments the respiratory chamber was cooled by surrounding it with ice. Sixteen rats were used in the first group of experiments in which only air was administered during artificial respiration. Five animals regained circulatory and respiratory function and 3 showed only a circulatory response.

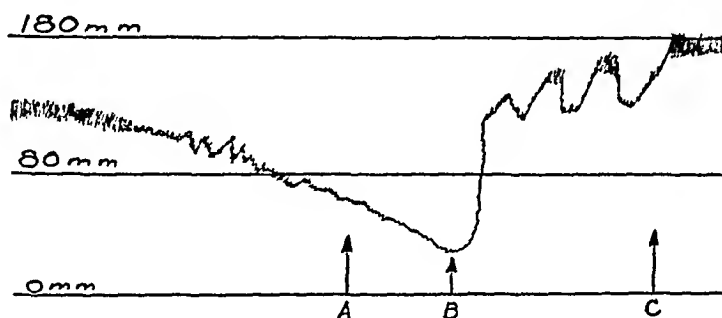


Fig. 5. CIRCULATORY RESPONSE DURING resuscitation with drinker respirator. Apnea began at A; artificial respiration administered at B; respiration resumed at C.

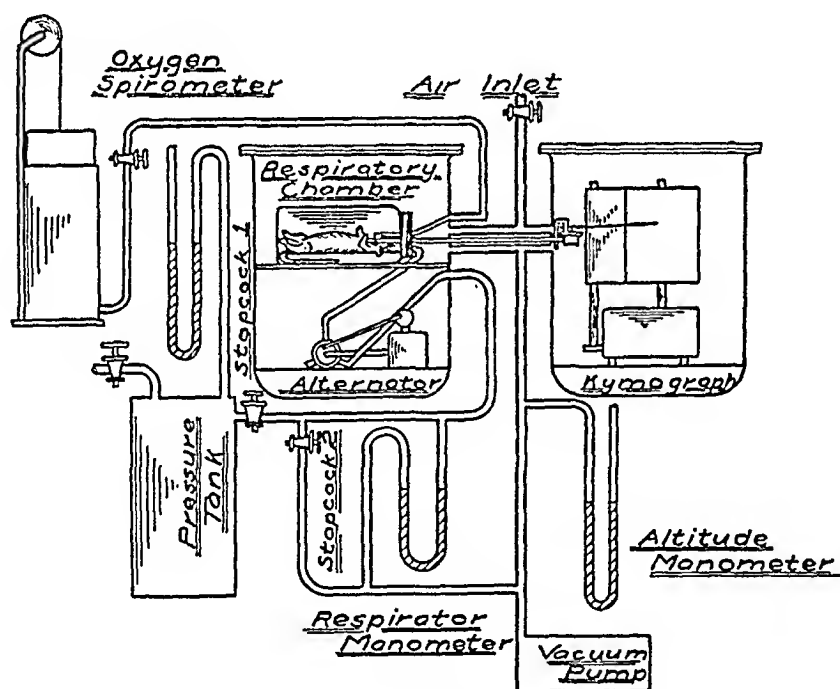


Fig. 6

In the second group of 12 rats an oxygen-carbon dioxide mixture was introduced and 6 of these animals completely recovered while 3 showed only a circulatory response. Figure 7 shows a typical blood pressure record during resuscitation. A study of the blood pressure records made on these animals indicated that the barospirometer produced a more rapid increase in the blood pressure than the other methods. With many animals, a circulatory response could be brought about on several successive trials which further demonstrated the beneficial effect on the circulation of this type

of artificial respiration. In all animals responding by this technic, the circulatory response preceded the respiratory response.

Respiratory Stimulants, High Altitude. Due to the manipulative difficulties associated with testing respiratory stimulants at high altitude, the only drug studied

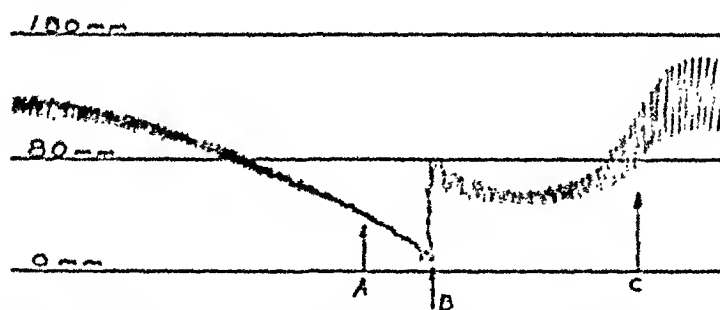


Fig. 7. CIRCULATORY RESPONSE DURING resuscitation with Barospirator. Apnea began at A; artificial respiration administered at B; respiration resumed at C.

TABLE 2

TYPE OF ARTIFICIAL RESPIRATION	ALTITUDE	NO. OF ANIMALS	CIRCULATORY RESPONSE		COMPLETE RECOVERY		CONDITIONS
			No.	%	No.	%	
Eve tilt board	Denver	10	0	0	0	0	Air
	High	9	0	0	0	0	
Schafer prone press.	Denver	10	0	0	0	0	Air
	High	15	6	40	6	40	
Pulmotor	Denver	12	9	75	8	67	Air O ₂ -CO ₂
	High	11	6	55	5	45	
	High	19	12	63	10	53	
Drinker respir.	Denver	12	8	67	7	58	Air O ₂ -CO ₂ Air, Refrig. O ₂ -CO ₂ , Refrig.
	High	9	0	0	0	0	
	High	3	0	0	0	0	
	High	11	5	45	5	45	
	High	15	11	73	8	53	
Barospirator	Denver	12	7	58	6	50	Air, Refrig. O ₂ -CO ₂ , Refrig.
	High	16	8	50	5	31	
	High	12	9	75	6	50	
Respiratory Stim. Coramine	Denver	6			6	100	
	High	7			2	29	

was the one that gave the best results at low altitude, namely Coramine. Seven animals were given Coramine intravenously when apnea due to anoxia developed but only 2 showed a respiratory response.

SUMMARY

The comparative results obtained with the various technics for artificial respiration are summarized in table 2.

At both high and low altitudes the mechanical methods for artificial respiration were superior to manual methods in the resuscitation of the rat. This is in line with the studies of Steinberg and Dietz and those of Swann on dogs.

The Schafer method was more effective at high altitudes than the Eve method. Cordier and Comroe and Dripps found the opposite of this in their studies on humans. Further, the Schafer method was 40 per cent efficient at high altitudes but none of the animals studied could be resuscitated at low altitudes. This suggests a greater recoil of the thorax and diaphragm at high altitudes than low which proves effective in the Schafer method but apparently offers no help with the Eve method.

The administration of oxygen while giving artificial respiration at high altitudes proves especially beneficial to the circulation. This was particularly true with the barospirator which was the most effective method studied for restoration of the circulation. Since the circulatory response always occurred first, this undoubtedly was important in bringing about the resumption of respiration.

A low environmental temperature facilitates resuscitation at high altitudes. It is evident from the results with the Drinker respirator that temperature had a greater effect than the administration of oxygen-carbon dioxide mixtures. None of the animals recovered with oxygen at the higher temperatures (34° – 35° C.) but 45 per cent of the animals studied at low temperatures (15° – 20° C.) showed complete recovery using only air.

Coramine proved effective in stimulating respiration at low altitudes but not at high. Other stimulants studied at low altitudes were caffeine, strychnine, picrotoxin, metrazol and potassium cyanide. Of these only strychnine approached coramine in effectiveness.

Rats were maintained for about 25 minutes by diffusion respiration using pure oxygen as the diffusion gas. Helium-oxygen mixtures offered no advantage over pure oxygen but in fact decreased the survival time of the animals.

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RELATIONSHIP BETWEEN HYPOXIA, OXYGEN CONSUMPTION AND BODY TEMPERATURE¹

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PREVIOUS work has apparently established the fact that in the hypoxia of high altitudes oxygen consumption is reduced and body temperature falls, (1-3). The environmental temperature is, however, a factor; at 37°C. there is no fall in body temperature nor in oxygen consumption but fewer animals survive a given degree of hypoxia (4). At least two questions of relationship are raised by these findings: 1) To what extent is oxygen consumption limited by oxygen availability? 2) Is the fall in body temperature the result of reduced oxygen consumption?

In the hope of obtaining information pertinent to these questions we have determined the oxygen consumption and body temperature of rats at five simulated altitude levels, 5280, 15,000, 25,000, 32,500 and 40,000 feet. The body temperature factor was varied by using three environmental temperature ranges, 18 to 20°, 26 to 28° and 35 to 37°C., and the metabolic factor by using normal, thyroxin-injected and thiouracil-treated rats:

EXPERIMENTAL

Young, adult, female rats, weighing between 175 and 250 grams, of the University of Denver strain were used. They are therefore rats acclimatized to an altitude of 5280 feet. Thiouracil treatment consisted in feeding meal into which 50 mg. of the drug per 100 gm. of meal had been thoroughly incorporated and in providing a saturated solution for drinking water. No determinations of food and water consumption were made, hence the dosage cannot be given, however, during the 3- to 5-week-period during which the rats were on this diet an increase in thyroid weight of approximately 300 per cent was achieved. Thyroxin administration consisted of the injection of 1 mg/kg. of Squibb's 'Thyroxin Fraction' every other day for a similar period of time. The rats used at the low and high environmental temperatures are the same rats, being run first at the low temperature; those at the moderate temperature are a different series. This was a compromise between using the same rats throughout, which would have been desirable, as against the fact that they would then have been under the influence of the drug for considerably different periods of time.

Oxygen consumption was determined in a closed system apparatus as shown in figure 1, and the body temperature by means of a copper-constantan thermocouple, inserted to a depth of 4 cm. into the colon. In making a test the rat was lightly

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anesthetized with 25 mg/kg. of sodium pentobarbital to eliminate struggling caused by the presence of the thermocouple, and placed in the chamber for 20 minutes before the first determination was made. During this time air was slowly drawn through the chamber by means of the vacuum pump. The chamber was then sealed by closing the screw clamps leading to the air inlet and the vacuum pump. A wick soaked in 5 per cent sodium hydroxide surrounded the rat and absorbed the carbon dioxide produced, the resulting fall in pressure being indicated by the water-filled 'chamber manometer'. Oxygen was admitted from the measuring spirometer practically as rapidly as it was consumed; there was therefore no significant change in the oxygen pressure within the chamber. The oxygen consumption was determined over a 10-minute period, whereupon the pressure was reduced by means of the vacuum pump, air being bled through the chamber at the same time. The rate of ascent was

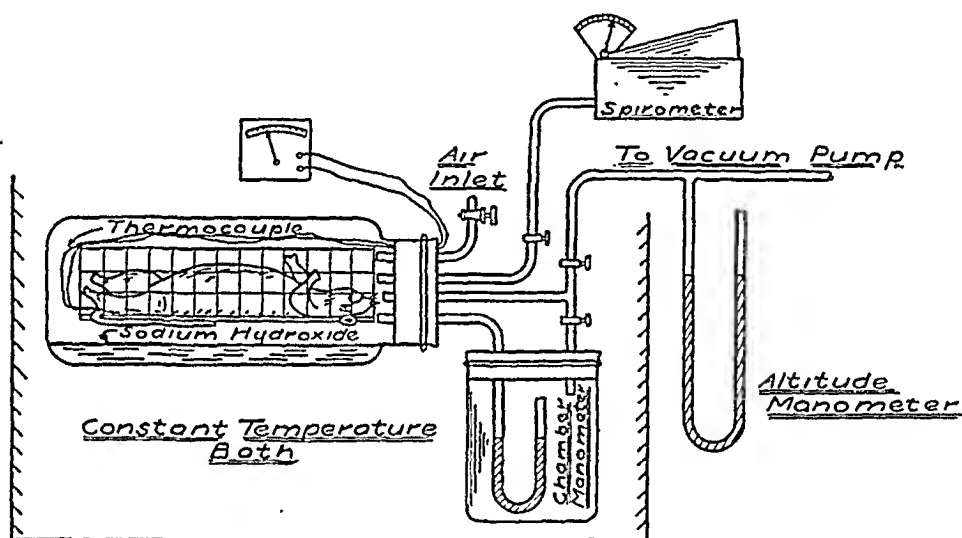


Fig. 1

uniformly 2000 feet per minute. When the desired elevation was reached the chamber was again sealed off and the test repeated as described. Each test was for a 10-minute period with the water manometer kept at practically a constant level by frequent admission of oxygen. Body temperature readings were taken at the end of each 10-minute run.

The results obtained are shown in the following tables and graph. It should be noted that in the high temperature group the elevation at which the record stops is the one reached by all members of the group. At least 70 per cent died either during the ascent to, or shortly after reaching, the next elevation. Each of the nine experimental groups consisted of ten animals.

DISCUSSION

We believe the data obtained are primarily of interest as showing how oxygen consumption and body temperature vary under the conditions of the experiment. Any conclusions as to relationships must be highly tentative as many uncontrolled factors are operative. Reduced oxygen availability resulting from a lowered baro-

TABLE I

	5280	13,000	25,000	33,500	40,000
<i>A. Oxygen Consumption in ml/kg/min.</i>					
	<i>Env. temp.—18–20°C.</i>				
Thyroxin.....	56.8	57.5	40.9	33.6	21.8
St. D.....	5.7	5.2	4.7	4.3	3.0
Normal.....	39.2	41.1	30.5	25.5	19.1
St. D.....	4.0	5.1	2.8	3.4	2.6
Thiouracil.....	38.2	46.7	29.0	18.8	15.4
St. D.....	5.1	3.3	4.6	3.6	2.0
	<i>Env. temp.—26–28°C.</i>				
Thyroxin.....	35.4	34.7	30.4	22.2	12.6
St. D.....	2.0	2.1	1.6	1.9	1.1
Normal.....	24.5	24.7	23.3	21.9	12.1
St. D.....	1.9	1.8	1.6	1.5	2.0
Thiouracil.....	18.0	18.2	18.6	17.0	14.9
St. D.....	1.5	2.2	2.0	1.8	1.2
	<i>Env. temp.—35–37°C.</i>				
Thyroxin.....	48.3	48.8			
St. D.....	6.0	3.8			
Normal.....	28.7	26.9	27.8	24.8	
St. D.....	3.1	2.6	3.4	3.1	
Thiouracil.....	24.5	25.8	27.1	18.8	
St. D.....	4.0	2.4	1.2	1.8	

B. Body Temperatures in °C.

	<i>Env. temp.—18–20°C.</i>				
Thyroxin.....	35.6	34.1	32.4	30.8	28.5
St. D.....	1.2	.8	1.0	1.2	.9
Normal.....	34.9	33.4	32.1	30.7	29.2
St. D.....	.8	1.0	.6	.6	.8
Thiouracil.....	33.2	32.1	30.4	28.6	27.1
St. D.....	1.3	1.1	1.0	.9	1.2
	<i>Env. temp.—26–28°C.</i>				
Thyroxin.....	37.2	37.1	36.7	35.6	34.3
St. D.....	1.0	.9	.4	.8	.6
Normal.....	36.1	35.5	35.1	34.5	34.0
St. D.....	.4	.7	.7	.5	.5
Thiouracil.....	34.8	34.3	33.5	32.9	32.1
St. D.....	.9	1.1	1.0	.7	.7
	<i>Env. temp.—35–37°C.</i>				
Thyroxin.....	38.7	39.8			
St. D.....	.6	.7			
Normal.....	37.9	39.1	40.0	40.5	
St. D.....	1.2	.8	1.0	.6	
Thiouracil.....	36.8	37.6	38.0	38.3	
St. D.....	.5	.5	.4	.5	

metric pressure may be compensated for by increased respiration, increased utilization, etc.; heat production may not depend solely on oxygen consumption, since anaerobic, exothermic reactions may contribute, and whether variations in heat production will affect body temperature will depend on the rate of heat loss.

Limiting the discussion to the data at hand and considering first the question of the relationship between oxygen availability and oxygen consumption, we believe that availability does not, in normal rats, become the limiting factor in consumption until altitudes approaching 40,000 feet are reached, but may become a limiting factor

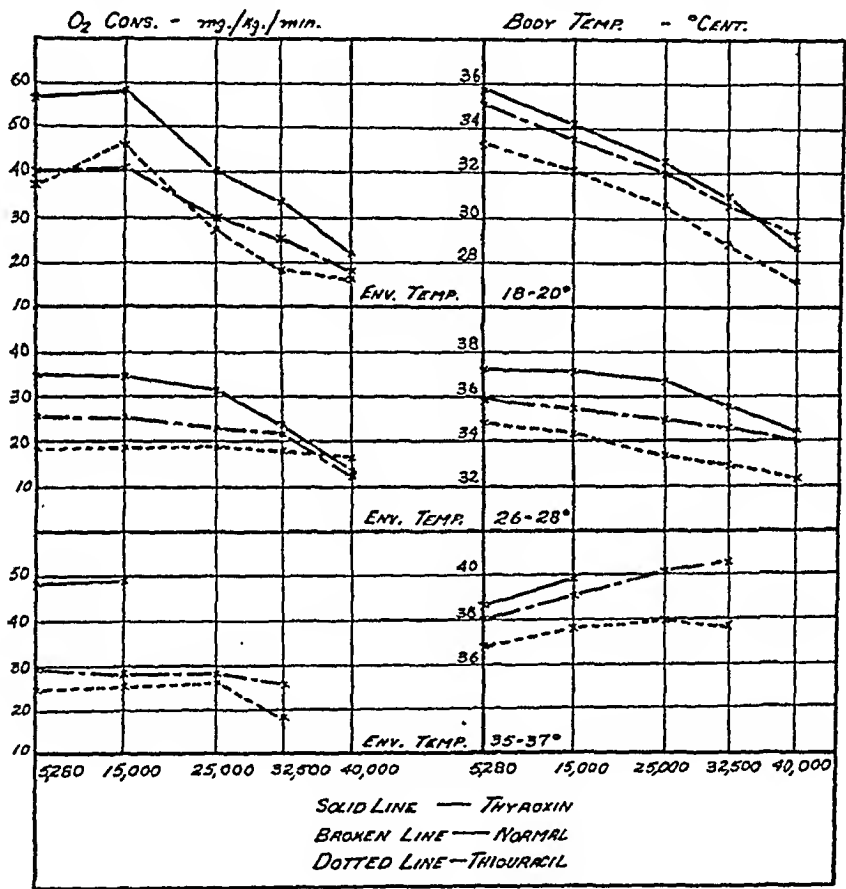


Fig. 2

at higher levels of availability in rats whose metabolism has been stimulated by cold or by thyroxin. This is indicated by the fact that the oxygen consumption of normal rats at moderate temperatures falls only slightly until an altitude of 32,500 feet is reached, the fall is steeper and begins at lower altitudes for the groups whose metabolism has been stimulated by cold or by thyroxin, and is steepest of all in the group whose oxygen consumption was highest, namely the thyroxin-treated, cold-environment group.

Although the close parallelism of the oxygen-consumption and body-temperature curves strongly suggests a cause-and-effect relationship, certain comparisons may be made which indicate that the two factors may vary independently. 1) In the low environmental temperature groups all oxygen consumptions at 40,000 feet are higher

than at moderate temperatures, the body temperatures, however, fall to much lower levels. 2) The oxygen consumption of the thyroxin-injected group at low environmental temperature is maintained well above the normal; the body temperature, however, falls just as precipitately. 3) The oxygen consumption of the thiouracil-treated group at moderate environmental temperature is practically constant; the body temperature, however, falls. 4) The oxygen consumption of the normal group, at high environmental temperature, remains practically constant (until death at 32,500 feet); the body temperature, however, rises.

Certain additional findings may be noted. 1) Thiouracil, in a dosage sufficient to enlarge the thyroid three-fold and to produce a reduction of 27 per cent in the oxygen consumption at moderate temperatures, does not depress the oxygen consumption significantly in the face of the stimulus of environmental cold, whereas the increases due to thyroxin and to cold are additive. 2) Thiouracil, although apparently preventing as great a rise in body temperature at high environmental temperature as occurred in the normal group, was not protective; all rats in both groups died at about the same time in going beyond 32,500 feet. Thyroxin, on the other hand, was detrimental, 7 of 10 rats dying between 15,000 and 25,000 feet. 3) It is difficult to explain the deaths of the normal and thiouracil-treated groups at high environmental temperature. The thyroxin group may well have died because of inability to supply their greatly increased oxygen demands, but the normal and thiouracil groups consumed, at 32,500 feet respectively, 24.8 and 18.8 mg/kg/min., not greatly different than the 21.8 mg. which the thyroxin-treated group was able to supply at 40,000 feet at low environmental temperature. The explanation may lie in the alveolar vapor pressure factor. There would be a difference in vapor pressure of 22 mm. (av. body temperature at low environmental temperature is 29°, v.p. 30 mm.; at high environmental temperature the av. body temperature is 39°, v.p. 52 mm.), which at 40,000 feet would give the low environmental temperature group approximately a 20 per cent greater alveolar oxygen pressure.

SUMMARY

The oxygen consumption and body temperature of normal, thyroxin and thiouracil-treated rats were determined at three environmental temperatures and five altitude levels between 5280 and 40,000 feet. The data obtained indicate: 1) Oxygen availability becomes a limiting factor in oxygen consumption only at altitudes approaching 40,000 feet in normal rats, but at much lower altitudes in animals whose metabolism has been stimulated by cold or by thyroxin. 2) Although a reduced consumption of oxygen is in general accompanied by a fall in body temperature, instances occurred where the two factors apparently varied independently of each other.

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FURTHER OBSERVATIONS ON THE DEPRESSION OF RENAL FUNCTION FOLLOWING HYPOPHYSECTOMY¹

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IT HAS been shown (1) that the considerable falls in sodium para-aminohippurate (PAH) and inulin clearances and in PAH Tm resulting from hypophysectomy in dogs are not due primarily to the resultant depression of thyroid or of gonad function, since the falls are much greater than those caused by thyroidectomy or castration, the latter procedure being in fact without effect. The possibilities remained that the falls were due either *a*) to depression of adrenal cortical function through loss of adrenocorticotrophic hormone (ACTH) or *b*) to the loss of some other anterior lobe principle not thyrotrophic or gonadotrophic. Two types of approach to a decision between these alternative possibilities are available.

The more direct is to establish an adrenal cortical replacement therapy adequate to maintain normal renal function in the adrenalectomized dog and to see whether such adrenal replacement therapy protects the hypophysectomized dog's renal function. If it does, it may be concluded that the falls in renal function following hypophysectomy are due primarily to the resultant depression of adrenal cortical function. If it does not, it may be concluded that the falls in renal function following hypophysectomy are not due to the resultant depression of adrenal cortical function, but to the loss of some further anterior lobe influence not thyrotrophic, gonadotrophic or adrenotrophic. If it is found that adrenal replacement therapy preserves the renal functions of the hypophysectomized dog, it should be shown that such therapy does not greatly raise the functions in the normal dog, i.e. that increases seen in the hypophysectomized dog are not due to overdosage.

The second approach is to produce the falls in renal function by hypophysectomy and to see whether the functions under observation can be restored to normal by treatment with 'pure' ACTH. If it is found that such treatment does restore the lowered renal functions of the hypophysectomized dog, such finding may be explained either on the basis that *a*) the restoration of adrenal cortical function brought the renal function back to normal, i.e. the fall in renal function after hypophysectomy was due primarily to the depression of adrenal cortical function through loss of ACTH; or *b*) the ACTH preparation employed contained the further anterior lobe principle referred to above and tentatively designated in our earlier report (1) as 'renotrophic' hormone. To distinguish between these two possibilities, the adrenalectomized dog whose renal function is maintained by adrenal replacement therapy may be given

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ACTH. If the renal functions under observation are raised by such ACTH treatment, this may be interpreted, provided the ACTH preparation is essentially free from thyrotrophic principle, as evidence in favor of possibility *b*; if they are not raised the evidence is in favor of possibility *a*.

If treatment with ACTH does not restore the observed renal functions of the hypophysectomized dog, it may be concluded that the falls in function resulting from hypophysectomy were not due to the loss of ACTH, but to the loss of some further anterior lobe influence and that the ACTH preparation employed does not exert such an influence. The present paper reports on experiments designed to answer the above questions and presents evidence that the falls in renal function observed after hypophysectomy are not due to depression of adrenal cortical function but to loss of an anterior lobe principle, not thyrotrophic, gonadotrophic or adrenotrophic, which directly influences the functional state of the kidney, or of the entire organism, in such a way as to result in increased renal activity.

METHODS

Trained female dogs in the postabsorptive state were used; the diet was dog chow *ad libitum* supplemented with meat every other day. Two or more control clearance and T_m experiments were run on each animal to establish normal baselines. Two dogs (*K40* and *K41*) were adrenalectomized, the left adrenal being removed at one operation and the right one week later; three pellets (75 mg. each) of desoxycorticosterone acetate (DCA)² were implanted subcutaneously at the first adrenalectomy. Three dogs (*K37*, *K39* and *K42*) underwent simple hypophysectomy. *K37* received four pellets and *K39* three pellets of DCA prior to hypophysectomy; *K42* received three pellets on the 41st day after hypophysectomy. All dogs also received additional adrenal cortical therapy by daily injection of whole cortical extract for a few days postoperatively.

Good urine flows for the experiments were induced by giving 3.5 per cent body weight of water about 25 minutes before the beginning of the first urine collection. A blank blood was drawn and immediately followed by a priming intravenous injection of 0.025 cc. of 20 per cent PAH/kg. and 0.05 gm. inulin/kg. in 5 cc. of 0.5 per cent NaCl³. This was followed by a subcutaneous sustaining injection of 0.11 cc. 20 per cent PAH/kg., 0.20 gm. inulin/kg. and 400 μ g. hyaluronidase⁴ (2), all diluted so that the inulin was present as a 10 per cent solution. The injection was made through a single skin puncture in the shoulder area, the direction of the needle being changed four to five times. The first clearance period began about 25 minutes after the end of the subcutaneous injection. These amounts of PAH and inulin produced plasma levels of 1 to 2.5 mg. per cent and about 20 mg. per cent, respectively. The plasma levels of PAH and inulin do not remain absolutely constant following this procedure, but the changes after the first 25 minutes are usually small and plotting the successive plasma levels against time permits an accurate estimation of mean levels for the various periods. Two 15- to 20-minute urine collections were made, the bladder being washed twice with 20 cc. normal saline at the end of each collection. Blood samples were drawn at the beginning and end of each clearance period. Immediately following the drawing of the last blood sample at the end of the second urine period, high plasma levels of about 20 mg. per cent PAH were attained by giving a priming intravenous injection of 0.25 cc. 20 per cent PAH/kg. diluted 1 to 4 with 0.5 per cent saline and a sustaining subcutaneous injection of 1.3 cc. 20 per cent PAH diluted 1 to 3 with water and including 400 μ g. hyaluronidase. The urine collections again began about 25 minutes after the end of the subcutaneous injection.

Hypophysectomized dogs, with lowered renal function, were given the same intravenous prim-

² The DCA was generously supplied by the Schering Corporation.

³ The PAH was generously supplied by Sharp & Dohme.

⁴ The hyaluronidase was generously supplied by the Schering Corporation. We are not yet able to state positively whether hyaluronidase significantly speeds the absorption of PAH and inulin.

ing injection for the clearance periods, but the subcutaneous injection was reduced to 0.08 cc. 20 per cent PAH/kg. and the inulin to 0.12 gm/kg. For the Tm the intravenous injection was 0.2 cc. 20 per cent PAH/kg. and the subcutaneous 0.5 cc/kg.

Inulin was determined by a modification of the method of Corcoran and Page (3) and PAH, by a slight modification of the method of Smith *et al.* (4). Both plasma PAH and inulin were determined on a zinc filtrate (5), requiring a correction for PAH since only 93.5 per cent appears in the filtrate. Recovery of inulin is complete. Attempts were made to recover inulin from a cadmium precipitation of plasma but the recoveries were not only incomplete but quite inconstant. We also tried making fine adjustments in the normality of the NaOH (carbonate-free) used in this precipitation but were not able to improve either the percentage or the constancy of the inulin recovery. Cadmium filtrates yield complete recoveries of PAH. Glucose was determined on all plasma samples according to Nelson (21) and correction was made for any errors in apparent plasma inulin values due to fluctuations in plasma glucose. Plasma sodium was determined according to Butler and Tuthill (18) or by flame photometry, using a burner and mixing chamber designed by Dr. Theodore Weichselbaum of the Surgical Metabolism Laboratory of this School or by both methods. Plasma potassium was determined according to Van Slyke and Rieben (19) or by flame photometry or by both. Plasma NPN was determined by a micro Kjeldahl digestion with direct Nesslerization. Urea determinations were according to Van Slyke and Kugel (20).

RESULTS

Adrenal replacement therapy. The first objective is to establish the adrenocortical replacement therapy which will support the observed renal functions of the adrenalectomized dog at or near the normal levels. We have previously found (1) that four 125-mg. DCA pellets subcutaneously implanted afford adequate replacement. In the present series we gave three 75-mg. pellets to each of 2 adrenalectomized dogs. For one dog (*K40*) this appeared to be adequate in spite of a severe cellulitis from Feb. 17 to March 7, 1948; the other dog (*K41*) failed to maintain completely normal renal functions, although its weight, appetite, general behavior and plasma sodium and potassium remained normal. Three 75-mg. DCA pellets appear to be at or just below the borderline required to maintain the clearances and Tm normal in the adrenalectomized dog; even with *K41* the renal functions were supported well above the levels seen after hypophysectomy with or without DCA. The results with the DCA pellets in the adrenalectomized dogs of the present series are given in table 1; the effects of ACTH are also given.⁵

Adrenalectomy. At two and at eight weeks after removal of the second adrenal, the renal functions of *K40* are essentially normal, with the PAH clearance actually higher than any of the pre-operative values. Plasma Na, K and NPN values at two weeks were normal; plasma Na remained normal thereafter but plasma NPN rose

⁵ The ACTH was generously supplied by Armour and Company. Three lots have been obtained, 32-D, 37-KE and 42-B; the manufacturer's specifications follow. The adrenotrophic potency per mg. of 32-D is 71% that of Armour's unofficial standard La-I-A; its growth hormone, gonadotrophin and thyrotrophin contents are negligible. A single intravenous injection of 0.004 mg. of Armour's standard La-I-A produces consistently a 20 to 30% decrease in the adrenal ascorbic acid content of the hypophysectomized rat; 0.004 mg. of standard will be designated as 1 u. Lot 37-KE has 50% of the adrenotrophic potency of Armour's standard, 0.5 u. prolactin/mg., 2.0 Collip u. of gonadotrophins/mg., and 0.037% Evans chick u. of thyrotrophin/mg. Lot 42-B has 16% of the adrenotrophic potency of Armour's standard, less than 1 u. prolactin/mg., less than 1.5 Collip u. gonadotrophin/mg. and negligible growth hormone. The posterior pituitary hormone content in all of these batches was negligible for our purposes. The dosages of ACTH given in the tables express the number of ACTH u. given 3 times daily, e.g. in table 1 from 1/5/48 to 1/13/48 dog *K40* was given every 8 hours an amount of lot 37-KE equivalent to 625 u.

TABLE I. MAINTENANCE OF RENAL FUNCTIONS IN ADRENALECTOMIZED DOGS

REMARKS. ACTH DOSAGES, AS EQUIVALENTS OF ARMOUR'S STANDARD, GIVEN EVERY 8 HOURS		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH TM	PLASMA Na & K NPN	
<i>Dog K₄₀</i>						
		cc/min/ M ²	cc/min/ M ²	ml/ min/ M ²	mg/L mg %	
11/ 3/47	Normal	184	74	15.3		
12/22/47	Normal	203	90	14.9		
1/ 5/48-1/13/48	625 U. ACTH (37-KE)					
1/12/48		302	106	20.5		
1/21/48	L. adrenalectomy; 3 DCA pellets					
1/28/48	R. adrenalectomy					
2/12/48		291	90	12.5	Na	146
	Severe cellulitis 2/17 to 3/7				K	4.44
					NPN	27
3/22/48		280	89	14.4	NPN	37
3/25-3/30	875 U. ACTH (32-D)					
3/29/48		361	126	11.6	NPN	37
5/ 6-5/13	240 U. ACTH (42-B)					
5/12/48		200	87	7.6	Na	144
					NPN	39
5/29/48					Na	143
					NPN	47
6/10/48	Plasma urea clear. 42 cc/min/M ²		71		NPN	48
	Plasma urea N 27 mg %					
6/15/48					Na	143
7/ 2/48	Plasma urea N 21 mg %. Dog killed same date	194	71	10.6	Na	141
					K	4.0
					NPN	41
<i>Dog K₄₁</i>						
10/27/47	Normal	354	120	19.3		
12/12/47	Normal	333	97	15.6		
1/ 7/48-1/15/48	625 U. ACTH (37-KE)					
1/14/48		313	108	36		
1/21/48	L. adrenalectomy; 3 DCA pellets					
1/28/48	R. adrenalectomy					
2/ 5/48		260	114		NPN	39
2/ 9/48		273	105	8.7	Na	146
					K	4.3
					NPN	43
2/24/48		213	86	12.3	Na	142
3/ 4-3/12	0.2 cc. (160 dog U.) Upjohn's lipo-adrenal cortex daily				K	4.8
					NPN	37
3/11/48		216	79	11.8	NPN	40
4/ 5/48		190	71	11.7	NPN	38
4/13/48					Na	143
5/11-5/18	400 U. ACTH (42-B)					
5/17/48		209	75	10.8	NPN	39
5/29/48					Na	140
					NPN	57

TABLE 1.—Continued

REMARKS. ACTH DOSAGES, AS EQUIVALENTS OF ARMOUR'S STANDARD, GIVEN EVERY 8 HOURS		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH Tm	PLASMA Na & K NPN
<i>Dog K41—Continued</i>					
		cc/min/ M ²	cc/min/ M ²	mg/ min/ M ²	mEq/L mg %
6/ 7/48	Plasma urea clear. 41 cc/min/M ² Plasma urea N 25 mg %		71		NPN 47
6/15/48					Na 143
7/15/48					NPN 42
7/23/48		192	67	9.4	Na 143
					NPN 46
7/28/48					Na 146
					K 3.1
8/ 2/48					Na 145
					NPN 43
8/27/48					Na 143
					K 4.4
9/13/48					Na 135
					K 6.0
					NPN 44
9/15/48					Na 146
					K 5.2
					NPN 44
9/17/48					Na 144
					K 6.3
					NPN 47
9/22/48					Na 145
					K 6.3
					NPN 37

slowly in spite of a well maintained inulin clearance. The renal functions of *dog K41* were less adequately maintained. Inulin clearance and PAH Tm had fallen only slightly at 74 days (4/5/48) after the second adrenalectomy but PAH clearance was considerably reduced below the control value. This dog, however, had unusually high normal PAH clearance values and even the lowest postadrenalectomy value of 190 is not greatly below the usual normal limits. The failure of the lipo-adrenal cortex (3/11/48) to improve this may mean that more prolonged treatment is necessary to re-establish a slightly depressed renal function or that administration once daily is not frequent enough. The daily dosage should have been more than adequate, since it was 8 rat units or 160 dog units, where a dog unit is the amount required daily per kilo of adrenalectomized dog to maintain normal body weight and blood NPN (6). In a third adrenalectomized dog (*K31*) previously reported (1), DCA pellets restored essentially to normal the depressed renal functions seen during adrenal insufficiency. Thus in 2 (*K31* and *K40*) of 3 adrenalectomized dogs, DCA pellets maintained renal functions essentially normal while in the third (*K41*) the protection was apparently less complete. The protective action of DCA pellets in adrenalectomized dogs is, of course, not permanent but lasts for several months. It

TABLE 2. FAILURE OF MAINTENANCE OF RENAL FUNCTIONS IN HYPOPHYSECTOMIZED DOGS

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH TM	PLASMA NPN
<i>Dog K37</i>					
		cc/min/ 3ft	cc/min/ 3ft	mg/ min/3ft	mg %
4/28/47	Normal	186	77	14.5	
5/ 7/47	Normal	192	66	15.5	
5/12/47	Normal	184	70		
5/13/47	4 DCA pellets of 75 mg. each				
5/22/47	Normal with DCA pellets	220	83	18.1	
5/22-5/30	150 u. preloban plus 6 mg. DCA in oil daily				
5/29/47		387	153	25.1	
6/ 4/47	Simple hypophysectomy				
6/18/47		157	66	6.7	
6/19-6/27	0.2 cc. (160 dog u.) Upjohn's lipo-adrenal cortex daily				
6/26/47		155	71	7.1	
6/27-7/4	440 u. ACTH (32-D)				
7/ 3/47		173	83	5.1	
9/15/47		168	53	7.1	
12/29/47		190	65	11.2	
1/ 2/48	Autopsy; fragment of anterior lobe tissue (about 5%) in sella				
<i>Dog K39</i>					
6/ 2/47	Normal 3 DCA pellets of 75 mg. each				
6/13/47	Normal with DCA pellets	264	95	18.7	
6/16/47	Normal with DCA pellets	263	96	22.9	
6/20/47	Simple hypophysectomy				
7/ 7/47		158	63	6.1	
7/8-7/15	0.2 cc. (160 dog u.) Upjohn's lipo-adrenal cortex daily				
7/14/47		168	72	6.5	
7/15-7/22	440 u. ACTH (32-D)				
7/21/47		247	91	15.3	
9/10/47		176	44	8.8	
12/26/47	2 pellet fragments (81 mg.) found & removed				
12/31/47		151	45	8.6	
1/12/48-1/20/48	625 u. ACTH (37-KE)				
1/19/48		183	61	9.9	
1/20/48	3 DCA pellets				
2/16/48		129	52	5.6	37
3/2-3/10	0.2 cc. (160 dog u.) Upjohn's lipoadrenal cortex daily				
3/ 9/48		143	55	8.4	45
5/5-5/11	240 u. ACTH (42-B)				
5/10/48		145	52	6.9	

TABLE 2.—Continued

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH Tm	PLASMA NPN
<i>Dog K42</i>					
		<i>cc/min/ M²</i>	<i>cc/min/ M²</i>	<i>mg/ min/M²</i>	<i>mg %</i>
11/10/47	Normal	247	77	22	
12/15/47	Normal	276	95	25.7	
1/ 8/48-1/16/48	625 U. ACTH (37-KE)				
1/15/48		254	94	16.2	
1/21/48	Simple hypophysectomy				
2/26/48		130	53	5.3	66
3/ 2/48	3 DCA pellets of 75 mg. each				
3/24/48		124	49	6.5	50
3/25-4/1	0.2 cc. (160 dog U.) Upjohns ¹ lipoadrenal cortex daily				
3/31/48		151	52	7.3	50
4/28/48		165	67		56
5/13-5/20	400 U. ACTH (42-B)				
5/19/48		158	61	8.6	40

will be seen in table 2 that this adequate or nearly adequate adrenal replacement therapy had no protective effect on the renal functions of hypophysectomized dogs. The results with ACTH administration will be discussed in a later section.

Hypophysectomy. In contrast with the findings in adrenalectomized dogs, table 2 shows that DCA pellets implanted before simple hypophysectomy (*K37* and *K39*) did not protect against the falls in renal function always seen after hypophysectomy, nor did implantation of pellets after the falls had been produced by hypophysectomy (*K42*) exert any restorative action. Supplementing the adrenal cortical therapy with large doses of Upjohn's lipoadrenal cortex also was without effect. On the basis of the degree of adequacy of such adrenal replacement therapy in adrenalectomized dogs (table 1), it seems quite improbable that the hypophysectomized dogs (table 2) could have been in adrenal insufficiency. The conclusion therefore seems justifiable that the falls in renal functions always seen after hypophysectomy cannot be due to adrenal insufficiency resulting from hypophysectomy. Since we have already shown (1) that the falls in renal functions following hypophysectomy are not due to loss of thyrotrophic or gonadotrophic hormones, it is concluded that such falls are due to loss of a 'renotrophic' hormone, where this term is used "in the sense of a substance formed in the pars distalis, essential to the normal maintenance of renal blood flow, glomerular filtration fraction and Tm, without commitment as to the mechanisms involved, which does not act through the mediation of other endocrine glands, but which may require the presence of some adrenal cortical hormone in order to be effective".

Dog K37 requires special comment. DCA pellets (6/18/47), even when supplemented by lipo-adrenal cortex (6/26/47), did not prevent the hypophysectomy from bringing about a considerable fall in renal functions below the prehypophysectomy

levels (5/22/47). There was subsequently some increase in values (12/29/47), presumably because of some regeneration of a remaining fragment of anterior lobe which was found at autopsy. In all the hypophysectomized dogs, serial sections of the tuberal region and decalcified sella or sellar contents were examined and in no case other than K₃₇ was any anterior lobe tissue found.

Normal. Table 3 shows that DCA pellets, even when supplemented with 6 mg. DCA in oil daily, do not significantly raise the observed renal functions in normal

TABLE 3. EFFECTS OF ENDOCRINE ADMINISTRATIONS TO NORMAL DOGS

REMARKS: ALL CLEARANCES AND Tm ARE PAH EXCEPT 3/29/46 OF THIS TABLE			PAH CLEAR- ANCE	INSULIN CLEAR- ANCE	PAH Tm
<i>Dog K₃₅</i>					
			cc/min/ M ²	cc/min/ M ²	mg/ min./M ²
3/29/46	Normal	Diodrast clearance and Tm	214	75	21.8
2/19/47	Normal	Nembutal anesthesia	238	85	21
2/24/47	Normal		268	107	20.3
3/ 6/47	Normal		279	87	
3/13/47	4 DCA pellets of 125 mg. each (Ciba) ¹				
3/27/47			262	83	23.8
4/4-4/11	6 mg. DCA in oil daily				
4/10/47			270	91	27.1
4/12-4/18	150 u. preloban plus 6 mg. DCA in oil daily				
4/17/47			345	121	30
4/23/47	Simple hypophysectomy; died				
<i>Dog K₃₆</i>					
4/ 1/47	Normal		278	91	18.8
4/21/47	Normal		226	73	19.4
4/30/47	Normal		237	75	
5/ 1/47	4 DCA pellets of 75 mg. each				
5/19/47			238	92	18.7
5/19-5/27	150 u. preloban daily				
5/26/47			396	138	28
5/28/47	Simple hypophysectomy; died				

¹ These DCA pellets were generously supplied by Ciba Pharmaceutical Products, Inc.

dogs except for some increase of Tm in K₃₅. To the 2 dogs of table 3 may be added K₃₇ of table 2, before hypophysectomy. Here the values for both clearances and Tm were somewhat higher (5/22/47) 9 days after implantation of DCA pellets than the averages of the three preceding normal values. Any increase in the observed renal functions from such DCA treatment in normal dogs is usually not present and when present is slight.

Discussion of results with adrenal replacement therapy. Two (K₃₁ and K₄₀) of 3 adrenalectomized dogs maintained on DCA pellets showed renal functions essentially the same as their normal pre-operative values. The 3rd dog (K₄₁) showed some fall after adrenalectomy but the values remained well above those seen after

hypophysectomy, with or without DCA pellets. Addition of whole cortical hormone to this 3rd animal did not have any greater effect on renal function than DCA alone. It is thus seen that the adrenalectomized dog maintained at normal plasma electrolyte levels usually shows normal clearances and Tm.

Hypophysectomized dogs receiving additional adrenal cortical hormone, either as DCA pellets or as pellets plus whole extract, show depression in renal functions as great as those seen after hypophysectomy without treatment. Loss of anterior lobe in the dog results in atrophy of the zona fasciculata and zona reticulata of the adrenal cortex, with but little effect on the zona glomerulosa (7, 8). Since no disturbance of water or electrolyte balance or of plasma electrolyte levels (1) follows loss of the anterior lobe in the dog, it seems a reasonable hypothesis, although other possibilities exist (9), that the zona glomerulosa is the principal site of formation of those hormones concerned with the maintenance of water and electrolyte balance. If the essential factor in maintaining adrenalectomized dogs in normal renal function is the maintenance of normal plasma electrolyte levels, it is not surprising that the hypophysectomized dog would fail to respond to DCA treatment, since its own adrenals are still able to fill this rôle.

It is thus seen that the depression of renal functions after simple hypophysectomy in the dog is not due to a loss of adrenal cortical hormones regulating water and electrolyte balance, since no such loss occurs. There is, however, after simple hypophysectomy a disturbance of carbohydrate metabolism but the depression of renal functions seen after hypophysectomy cannot be due to the loss of such carbohydrate-regulating hormones as are formed in the adrenal cortex, even though such loss occurs. The evidence for this statement is threefold: *a*) the depression of renal functions following adrenalectomy can be prevented or relieved by DCA alone, *b*) the depression of renal functions following hypophysectomy cannot be prevented or relieved by the addition of whole cortical extract to DCA and *c*) stimulation of the adrenal cortex of the hypophysectomized dog with ACTH does not relieve the depression of renal function; the enhancement of renal function sometimes seen on giving ACTH to the hypophysectomized dog cannot be ascribed to adrenal cortical stimulation, since it is also sometimes seen on giving ACTH to the adrenalectomized dog and must therefore be ascribed to the presence of some further principle in the ACTH preparation.

Since the depression of renal function following hypophysectomy cannot be ascribed to thyroid, gonad or adrenal regression, it must be due to the loss of some anterior lobe principle which acts directly on other than endocrine tissues; this action may be in part on the kidney and in part on tissues in general, in such a way as to bring about increased renal activity. It cannot be solely a manifestation of the reduction in cardiac output and oxygen consumption, since both of these functions fall almost as much after thyroidectomy as after hypophysectomy (1), while the depression of renal function after thyroidectomy is relatively slight (1) as compared with that after hypophysectomy. It cannot be related to a fall in blood volume, since this is unchanged by hypophysectomy (11); we have also found that plasma volume by the dye method is unchanged by thyroidectomy. We believe that use of the term 'renotrophic hormone' is premature unless it is clearly understood that there is no implication that the substance exerts a trophic effect specifically on the kidney. The

effect may be on the body tissues in general, resulting indirectly in increased renal activity. It would perhaps be better simply to refer to an anterior lobe principle having a renotrophic effect. Whether the principle under discussion may be growth hormone is considered in the next section.

The point may be raised that the fall in renal function following hypophysectomy, while not due either to thyroid or to adrenal depression alone, may be due to their simultaneous occurrence rather than to the loss of some further anterior lobe principle. For several reasons we believe that this explanation can be excluded. First, the factors operating to maintain the renal function well above the levels seen after hypophysectomy can still operate in the complete absence of thyroid, since thyroidectomy has much less effect than does hypophysectomy (1). Second, the hypophysectomized dog given adequate adrenal replacement therapy has little or no adrenal deficiency (so far as maintenance of the observed kidney function is concerned) and certainly has less thyroid deficiency than does the thyroidectomized dog, yet his renal functions are much more depressed. Third, preloban, a multiprinciple anterior lobe extract, has a greater enhancing effect on renal function in normal and in hypophysectomized dogs than does thyroid administration (12). This effect of preloban cannot be through adrenal cortical stimulation, since adrenal cortical therapy does not increase the renal functions in either normal or hypophysectomized dogs (12 and present paper). Fourth, preloban acts other than through the thyroid, since it raises oxygen consumption and cardiac output in thyroidectomized dogs (1) and sometimes raises renal functions in thyroidectomized dogs (1).

Results with ACTH. The second approach has been to see whether the falls in observed renal functions following hypophysectomy can be prevented or relieved by ACTH. Dog K37, table 2 (7/3/47), showed no response to ACTH after hypophysectomy; before hypophysectomy (5/29/47), it had shown a large response to preloban⁶, a multiprinciple anterior lobe extract. Dog K39 after hypophysectomy showed a good response to ACTH on one occasion (7/21/47), a questionable response on another (1/19/48) and no response on a third (5/10/48). Dog K42 showed no response to ACTH before (1/15/48) or after (5/17/48) hypophysectomy. Thus in five runs on 3 hypophysectomized dogs, ACTH gave no response in three, questionable in one and good in one.

Table 1, however, shows that in one (Dog K40, 3/29/48) of three runs on adrenalectomized dogs, ACTH also gave a response; this shows that the response when present in hypophysectomized dogs need not be ascribed to adrenocortical stimulation and indicates the presence of some further anterior lobe influence.

Electrolyte, NPN and urea findings. Table 1 shows that DCA pellets maintain normal Na and K plasma levels in adrenalectomized dogs for several months and that the rise in plasma NPN is the earliest observed sign of incipient adrenal insufficiency, a fall in PAH Tm being the next most sensitive indicator. The rise in NPN begins while inulin clearance is still normal; this had also been observed in Dog K31 (1). It was expected that a diminished urea/inulin clearance ratio would be seen, but the experiments of June 10, 1948 on K40 and of June 7, 1948 on K41 show urea/inulin clearance ratios of 0.59 and 0.58, respectively, which are normal values.

⁶ The preloban was generously supplied by the Winthrop Chemical Company.

DISCUSSION

The finding with the first type of experiment, that adrenal replacement therapy adequate to maintain the observed renal functions of adrenalectomized dogs at or near the normal levels has no protective effect against the falls of these renal functions in hypophysectomized dogs, is interpreted as meaning that the falls seen after hypophysectomy are not due to adrenal insufficiency and are due to the loss of an anterior lobe influence not thyrotrophic, gonadotrophic or adrenotrophic, since these first two have previously been excluded (1). The finding with the second type of experiment, that the ACTH preparations employed had no effect on the renal functions of hypophysectomized dogs ascribable to adrenocortical stimulation, supports this interpretation. The anterior lobe principle whose loss is principally responsible for the depression of renal function following hypophysectomy is not further identified; the view that the anterior lobe may directly affect tissue metabolism has been expressed by earlier workers (13-17).

The question of growth hormone may be raised. The ACTH preparations employed gave positive effects on renal function only rarely in normal, in adrenalectomized and in hypophysectomized dogs; all are stated to have a negligible content of growth hormone. It is possible that quite small amounts of growth hormone are enough to account for the inconstantly observed effects. It is also possible that some further anterior lobe principle acting on the kidneys or on tissues in general is responsible; further experiments with known dosages of growth hormone should help to answer this.

SUMMARY

The depression of renal function in the dog following hypophysectomy is not due to adrenocortical regression dependent on loss of adrenotrophic hormone; two arguments are offered. First, adrenal replacement therapy adequate to maintain the renal function of adrenalectomized dogs at or near normal has no protective effect on the renal function of hypophysectomized dogs. Second, ACTH has no effect on the renal function of hypophysectomized dogs ascribable to adrenocortical stimulation. Since the thyrotrophic and gonadotrophic hormones have previously been excluded, the depression of renal function following hypophysectomy is ascribed to the loss of some further anterior lobe influence which acts on the kidneys or on tissues in general; the question of whether this is growth hormone is left unsettled.

Plasma urea and NPN begin to rise in adrenalectomized dogs maintained on DCA pellets while plasma Na and K and plasma inulin clearance are still normal; the expected fall in urea/inulin clearance ratio under these circumstances was not seen.

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NEPHRO-OMENTOPEXY, COMPENSATORY RENAL HYPERFUNCTION, AND PARALLEL MEASUREMENTS OF RENAL DYNAMICS

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THIS began as a study of the effect of omentopexy on renal dynamics. As a sequel, dynamics of each kidney before division of a renal artery and then of the remaining kidney have been measured. So far as known such studies have not been made before.

Nephro-omentopexy and other nephropexies. Claims of improvement of renal function after nephro-omentopexy in renal-vascular disease are questionable due to inadequate measurement of such function. Müller and Cholzow (cf. 1) and Ritter (2) reported favorably on few cases but Burger and Carter (3) and Weeks *et al.* (4) performed nephro-omentopexy, nephro-myopexy, or spleno-renopecty on 12 hyperpiesic² patients without lasting fall in arterial pressure.

Siter (1) stripped the renal capsule of cats and dogs and wrapped the kidney with omentum. He claimed that the kidneys functioned adequately even after their vessels were subsequently tied off. Kawashima imbedded a kidney in the abdominal muscles and later tied off both renal arteries; the dog survived (cf. 1). Paunz (5-8) decapsulated the kidney, halved it almost to its pelvis, inserted part of the omentum into the wound and enveloped the kidney with redundant omentum. In one case the renal artery was ligated and the opposite kidney removed. A year later the dog was killed and it was claimed that the renal artery was totally obliterated.

MacNider and Donnelly (9) and Davis and Tullis (10), using essentially Paunz' method, found that the intra-renal omental fat disappeared and was replaced by connective tissue and that capillary invasion into the kidney was not significant around the glomerular tufts. Apparently nobody since Paunz has maintained a kidney solely on blood supplied from an omento-, myo-, or splenopexy. Mansfield *et al.* (11) made dogs hyperpiesic by renal artery constriction and noted return of hyperpiesia within 40 days after nephro-omentopexy.

Compensatory renal hyperfunction. Two thirds of the canine total renal mass can be removed at a time without causing death. If three quarters of the total renal mass is removed survival is impossible. Destruction of the renal nervous plexus makes no difference (12). When compensatory hypertrophy of a canine kidney has occurred after removal of its fellow, half of the remaining kidney may be removed without fatal outcome (13). Compensatory hypertrophy is not a formation of new nephrons but is a hyperplasia and hypertrophy of tubular epithelium and glomerular tufts and, somewhat, an engorgement of blood-vessels (14-20).

After unilateral nephrectomy in rabbits the halved urea-clearance gradually rose to 66 per cent of normal (18); in dogs the urea-clearance became normal in 11 days after unilateral nephrectomy (17). Foster (21) found the urea-clearance approximately normal in patients who had sustained unilateral nephrectomy. Ellis and Weiss (22) arrived at similar results with phenol red excretion.

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² 'Hyperpiesia' is preferred to 'hypertension' because the former is derived exclusively from Greek and thus is etymologically acceptable.

METHODS

Exteriorization of the vesical trigone is described elsewhere (23). It was necessary to perform this operation to measure the function of each kidney separately in unanesthetized trained dogs.

Nephro-omentopexy. Three types of nephro-omentopexy were performed. In all types, the left kidney and the omentum were delivered through a lateral subcostal incision after splitting the external oblique, internal oblique and transverse abdominal

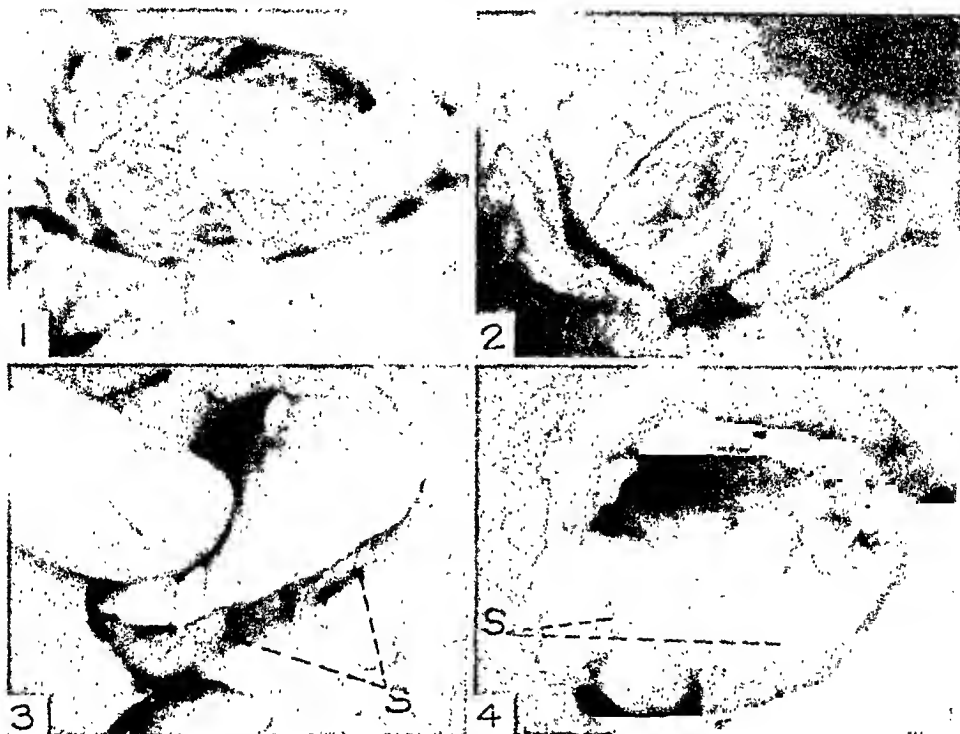


Fig. 1. KIDNEY DELIVERED through lateral subcostal incision two months after a *Type II* omentopexy. The light-colored broad band along the greater curvature of the kidney is where the omentum has been tucked into the deep coronal incision in the renal parenchyma.

Fig. 2. LATERAL ASPECT of kidney delivered through lateral subcostal incision. Two months after a *Type II* omentopexy.

Fig. 3. KIDNEY TWO MONTHS after a *Type II* omentopexy. The adherent omentum has been dissected away. The deep groove, S, is the site of the incision into which the omentum had been tucked. Note its smooth well-rounded surface.

Fig. 4. SAME KIDNEY as in fig. 3 but split open to show, more strikingly, the smooth, well-rounded surface which represents the cut surface at the time of the omentopexy.

muscles. In all, the kidney was decapsulated. The omentum was sutured to the portion of the capsule remaining at the hilum. Care was taken to apply the omentum to the kidney without torsion or tension and with minimal compromise to its blood-supply. In the *Type I* omentopexy the omentum was folded over the kidney in three layers, longitudinally, like a Japanese fan. The tail of the omentum was then folded over the kidney making a fourth layer. In the *Type II* omentopexy a rubber-shod bulldog clamp was applied to the left renal artery from the posterior aspect and the kidney was divided along its coronal plane almost to the renal pelvis. The medial border of the omentum was tucked all the way into the incision and kept in place by

three loose mattress sutures of fine 4-0 silk which were passed deep into the renal parenchyma. Three stab-wounds were made into each half of the divided kidney, which was then completely covered by folding over it the distal portion of the omentum. The bulldog clamp was removed in not over 25 minutes. Grasping the kidney with the fingers for a few minutes prevented bleeding. The kidney was returned into the abdominal cavity which was closed in layers. When such a kidney was re-exposed after several weeks for division of its artery, it appeared as in figures 1 and 2. The enveloping omentum continued to have a rich blood supply. The *Type III* omentopexy was similar to the first except that, in addition, the renal cortex was shaved throughout to a depth of about 0.25 cm.

Division of the renal artery and vein was made at least eight weeks after the omentopexy through a lateral subcostal incision adjacent to the scar of the former incision. The kidney and adherent omentum were carefully delivered through the wound and the renal artery and vein exposed by deflecting the kidney medially. The vessels were doubly ligated with 2-0 silk and divided. The operation was practically bloodless.

Renal denervation is related elsewhere (24). Proof of denervation was by epinephrine (see RESULTS). In all the operations anesthesia was by intravenous sodium pentobarbital, 32 mg/kg. The skin was shaved and prepared with acetone and aqueous zephiran, 1:500. Strict asepsis was practiced.

Renal clearances. Water was given by stomach tube, 50 cc/kg., and the clearances measured during diuresis. The creatinine clearance was used as a measure of glomerular filtration, which is permissible in the dog (25). Three gm. of creatinine, dissolved by heat in 30 cc. of saline and allowed to cool to body temperature, were injected subcutaneously bilaterally in the lateral abdominal area about 20 minutes before taking the first blood sample. The level in the plasma was fairly constant during the period of measurement, which lasted 15 to 20 minutes. The creatinine clearance was measured during the period of PAH clearance at low plasma levels of PAH and again during the period of measurement of tubular maximum for PAH. Four blood samples were taken in all; the first two were venous and the second two arterial. The initial creatinine clearance was taken as the value of glomerular filtration in table 1. The creatinine clearance immediately after injection of the large dose of PAH averaged only 59 per cent of the initial but was, necessarily, the value of filtration used in determining the tubular maximum for PAH. In only three of the many measurements was the second creatinine clearance equal to or slightly greater than the first. The drop in creatinine clearance, after the large dose of PAH, occurred regardless of whether the urinary flow was falling, rising or constant; it also took place in denervated kidneys and when arterial puncture was not done.

The effective renal blood flow was measured by adding 1.1 to 1.7 cc. of 20 per cent sterile sodium para-aminohippurate (product of Sharp & Dohme) to the above solution of creatinine. The plasma level was fairly constant during the 5 to 10 minutes of measurement and was always less than 3 mg/100 cc. (table 1). Collection of urine was directly from the external ureteral orifices (23). For measurement of maximal tubular transport of PAH, 25 to 35 cc. of the 20 per cent sterile solution were injected fairly rapidly into an external jugular vein. The third blood sample

TABLE 1

DOG; WT. Kg.	CONDITION	DAYS AFTER BLADDER EXTE.	GLOMERULAR FILTRATION CC/MIN.		EFFECTIVE RENAL BLOOD-FLOW CC/MIN.		PARI TM MO/MIN.		PARI PLASMA CONCENTRA- TION mg./100 cc.	
			L	R	L	R	L	R	Clin.	Tr.
A 16.2 to 16.4	Control L. nop.	8	34.4	37.6	158	172	24	27	0.73	176
		66	22.5	31.9	129	182			0.78	
B 12.9 to 11.1	Control L. nop.	7	32.0	37.2	201	179	25	26	0.55	174
		52	50.1	62.7	194	274	17	28	0.90	208
C 8.6 to 9.3	Control	15	30.3	25.6	141	122	12	13	0.72	221
	L. nop.	81	14.5	17.7	64	87	3	12	1.41	183
	L. renal art. divided ¹	95		29.5		253		24	0.79	246
		135	1.3	43.5	9	308		9	0.91	240
		184		58.9		438		24	0.76	181
D 11.3 to 12	Control	11	36.6	33.7	218	218	17	17	0.77	168
	L. nop.	25	22.1	35.4	103	149	10	14	0.75	165
		74	18.2	19.3	80	103	3	2	0.69	266
	L. renal art. divided	84	0	33.7	0	174	0	14	0.87	180
E ² 16 to 17.5	Control	8	68.5	55.5	267	319	12	16	0.87	180
	L. nop.	77	42.5	49.5	105	141	15	16	1.38	105
	L. renal art. & v. divided	92	0	79.7	0	497	0	47	0.90	187
		119	0	93.2	0	486	0	18	0.46	141
		134	0	92.5	0	798	0	64	0.47	168
		168	0	87.0	0	512	0	59	0.70	165
	R. kidney denerv.	183	0	98.0	0	761	0	76	0.90	158
F 12.2 to 12.5	L. nop.	8	26.1	38.3	114	167	8	10	0.47	133
	1 Branch of bifurcated l. renal artery divided									
		17	10.5	51.2	46	230	6	28	1.07	138
		26	10.1	38.1	53	198	7	47	0.79	144
	R. nephrectomy	35	30.9	0	110	0	11	0	1.01	182
G 12.7, 12.6	R. nephrectomy	59	28.2	0	133	0	20	0	0.91	152
G 12.7, 12.6	Control	6	42.1	23.9	234	141	18	17	0.70	177
		11	46.6	39.4	248	204	30	27	0.74	145
	L. nephrectomy	20	0	50.2	0	299	0	12	1.94	207
	L. nephrectomy	46	0	35.1	0	226	0	8	1.12	158
H 8.9, 9.1	Control	7	36.9	38.2	137	142	13	17	0.83	260
	L. kidney denerv.	17	31.4	28.2	126	113	7	10	1.21	321
	adrenalin, 22 mg/kg.	20	35	32	157	149			1.86	
			4	24	18	107			1.86	
	adrenalin, .011 mg/- kg.	22	32.5	28.5	135	138			1.79	
			25.7	22.2	130	102			2.06	
	R. nephrectomy	28	33.8	0	213	0	17	0	1.56	250
	R. nephrectomy	35	48.9	0	250	0			1.20	

L. nop. = left nephro-omentopexy.

¹ L. renal artery divided except for small branch to kidney.² An active young pure-blooded pointer.

was drawn from a femoral artery after about one minute. The urine was collected for the five minutes immediately after the third blood sample. The fourth blood sample was drawn from the femoral artery without delay. For the arterial punctures, a no. 20 Lindemann needle was used and kept in place during the six minutes, or the artery was exposed through a small incision under local 2 per cent procaine and a no. 22 hollow needle used. This was done under as sterile precautions as possible. Bleeding from the puncture was prevented by local compression after withdrawing the needle. The average concentration of PAH in the plasma was assumed to be the mean of the two blood samples for the second collection period (table 1). The concentration of each sample averaged ± 37 mg/100 cc. from the mean. The above assumption is doubtless inaccurate but, because of constancy in timing and in the amount injected into every animal, our Tm's probably have comparative if not absolute value. Analysis was after that detailed by Smith *et al.* (26). One-cc. pipettes were never used for delivering blood or urine.

RESULTS

Nephro-omentopexy reduces the rates of glomerular filtration, effective renal blood flow and maximal tubular transport (table 1: dogs A, B, C, E, F). Dogs A and E had a *Type I* omentopexy; the others had a *Type II*; and some which are not listed had a *Type III*.

Division of the main stem of the renal artery on the side of the omentopexy, over two months after the omentopexy, resulted in complete cessation of all renal function on that side (table 1: dogs D and E). This was also true in dogs with a *Type III* omentopexy. When a small renal branch of the renal artery was overlooked suppression of urine from the corresponding kidney was not complete (dog C). When the principal renal artery bifurcated into two main branches and only one was divided renal function on that side was approximately halved (dog F).

Nephro-omentopexy was thus valueless as a method of increasing renal function. On the contrary, due to the inevitable trauma of the procedure there was appreciable reduction of renal function; this, as would be expected, was most marked in the *Type III* omentopexy. The omentum retained its vascularity (figs. 1 and 2) and was closely adherent to the kidney, as it had been placed at operation, by fibrous tissue and, very sparsely, by small blood vessels. A new renal capsule was formed from fibrous tissue within a few days and developed no striking vascular connections with the omentum. The cut surface of the kidney, into which omentum had been tucked, became smooth and well rounded by fibrous tissue (figs. 3 and 4, S) and had only slight vascular connections with the omentum. After division of the main renal artery the omentopexy did not save the kidney from atrophy and, as seen in histological preparations, by fibrotic replacement.

The potentiality of nephro-omentopexy was tested to the limit in a six-month-old pup with a bifurcating left renal artery (table 1: dog F). Only one branch of the renal artery was divided eight weeks after omentopexy. Several weeks later the opposite kidney was removed. The animal's life thus hung on the hypertrophied one-fourth of its original renal mass. It continued to be healthy and have a voracious appetite. When, a few weeks later, the remaining branch of the left renal

artery was divided the animal promptly went into anuria and had to be killed on the third day because it had uremic twitchings. The omentopexy thus failed to support the kidney even when given the possible stimulus of partial renal ischemia for several weeks. Incidentally, the remaining branch of the left renal artery had enlarged markedly.

After division of one renal artery there was no rise in blood urea. This was evidently due to a practically prompt rise in the rates of glomerular filtration, effective renal blood flow, and maximal tubular transfer in the contralateral kidney (table 1: dogs C, D, E, F). The same applies after unilateral nephrectomy (dogs F, G, H). The operations were done carefully with practically no blood loss.

Rise in function of the contralateral kidney became obvious directly after the operation for division of the renal artery by an increased urinary flow from the ureter of the functioning kidney. The first actual measurements of renal function were made two days after division of the renal artery and showed a marked increase. There was a steady rise in the rates of glomerular filtration, effective renal blood flow, and maximal tubular transfer until about the end of the second month after which compensation stabilized (table 1: dogs C, G, H).

Compensation by augmented renal function of the intact kidney was possible even when renal function was depressed bilaterally presumably due to retrograde infection (table 1: dog D, after division of the left renal artery).

Denervation of a kidney did not prevent compensatory hyperfunction by that kidney after removal of its mate (table 1: dog H). Furthermore, denervation did not reduce already acquired compensatory hyperfunction (dog E). Complete denervation was demonstrated by the marked potentiation to a large intravenous dose of epinephrine (0.22 mg/kg.) by the denervated kidney compared with the contralateral control and the lack of potentiation to a much smaller dose of epinephrine (dog H). Potentiation of the denervated canine kidney to a large dose of epinephrine has been described by Kubicek *et al.* (27) who, however, did not exteriorize the vesical trigone and therefore did not make simultaneous comparison of a denervated kidney with its normally innervated mate. Incidentally, since denervation does not potentiate the kidney to a small dose of epinephrine, these results do not, in themselves, contra-indicate thoraco-lumbar sympathectomy for arterial hypertension.

DISCUSSION

Nephro-omentopexies do not augment renal function but rather reduce it. Furthermore, a kidney cannot be maintained solely on the meager adventitial blood supply provided by omentopexy. The few successful reports in the past were probably due to the over-looking of a branch of the renal artery. One of our dogs was kept alive and in health even after dividing a branch of a bifurcating renal artery and, several weeks later, removing the opposite kidney (dog F). It was thus living on a *hypertrophied* one-fourth of its renal mass.

The most interesting factor is the rise, abrupt, in renal dynamics of a kidney after removal of its mate or after division of the main artery of its mate. That compensatory hyperfunction is not affected by the nerve supply is shown here. Hyper-

trophy fails to occur after removal of the hypophysis in frogs (28a and b) and dogs (29-31a) and probably, specifically, of the anterior hypophyseal lobe (31b). Moreover, hypophysectomy strikingly reduces glomerular filtration, effective renal blood flow and maximal tubular transfer of otherwise normal dogs. There is no reduction in circulating blood volume. Loss of pitressin-forming tissue does not affect these renal functions. Conversely, hypophyseal extract produces a rise in the above renal functions in normal and in hypophysectomized animals. Thyroid gland, orally, results in similar although less marked increase in renal function (32).

SUMMARY

It was not possible to augment renal function by any type of nephro-omentopexy. On the contrary, renal function was reduced by the procedure. Nephro-omentopexy did not prevent renal atrophy after subsequent division of the renal artery. There was a marked and almost immediate rise in function of the contralateral kidney after division of one renal artery or after nephrectomy. Maximum hyperfunction was attained at the end of about two months. A dog was capable of living on a *hypertrophied* one-fourth of its renal mass. Already acquired renal hyperfunction was not inhibited by denervation. Hyperfunction of one kidney as a result of removal of its mate was not prevented by initially denervating that kidney. Even an infected kidney with reduced function underwent hyperfunction after its mate was removed or strangulated.

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EFFECT OF INTRAVENOUSLY ADMINISTERED GLUCOSE ON FOOD INTAKE IN THE DOG

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AS A man or animal ingests a meal the further intake of food is progressively inhibited. This obvious event of daily experience cannot be adequately explained physiologically. We do not know the mechanisms by which the ingestion of food brings about a reversal of the desire to eat, nor do we know the nature of the stimuli which produce this effect, nor where these stimuli act. We believe that undue emphasis has been placed upon the gastric factor in the regulation of food intake (1-5) and that other aspects of this regulatory mechanism require study.

The present report deals with one facet of a systematic analysis of the factors which might be concerned in the physiological regulation of food taking behavior by studying the effect of parenterally administered foodstuffs on food intake. We (6) have recently shown that intraperitoneal injection of glucose and protein hydrolysate mixtures in the rat had no more inhibitory effect on food intake than corresponding volumes of saline. The continuous eating habits of the rat and the difficulties in administering parenterally more than 25 per cent of the daily caloric requirement to this species prompted the present study on the effects of intravenously administered glucose, one of the products of normal digestion, on food intake in the dog. Controlled studies on this subject are not available.

Three series of experiments were performed. In the first, large amounts of glucose solution were administered during one-hour periods before feeding; in the second, glucose was infused for longer periods of time at rates the dog is considered capable of metabolizing and in amounts which formed significant portions of the daily caloric requirement; in the third, more concentrated glucose solutions were injected rapidly intravenously just before the dogs were allowed to feed.

METHODS

Five mature mongrel dogs were employed. The animals were housed in individual cages in an air-conditioned, relatively constant temperature dog room. They were fed daily by the same individual, and at the same hour. These animals had been employed in previous studies on food intake, were accustomed to the feeding procedures, tolerated intravenous infusions for long periods of time without restraints or restlessness, and were generally stabilized as to food intake and body weight.

The diet during the first and second series of experiments consisted of a commercial dried dog food (Pard, Dehydrated, Swift) moistened with cooked meat juice. One gram of this mixture contained 2.5 calories. In the third series of experiments a complete commercial dog food (Pard, Regular, Swift) was used; this contained 1.2 calories per gram of food. The materials infused were dissolved in pyrogen-free water at room temperature. No nausea, vomiting or fever occurred.

In the first and second series of experiments, the food was offered to the dogs 20 minutes after the completion of all intravenous infusions, and the pans were allowed to remain in the cages for exactly 45 minutes, following which they were removed and weighed. There was free access to water at all times. In the third series, the intravenous injections were performed directly prior to feeding.

RESULTS

In the first series, summarized in table 1, 2 dogs received 200 cc. of 20 per cent glucose in 0.3 per cent NaCl intravenously, one dog received 200 cc. of 10 per cent in 0.3 per cent NaCl and 2 dogs received 400 cc. of 20 per cent glucose in 0.3 per cent

TABLE 1

DOG NO.	PERIOD OF STUDY	NO. OF DAYS	AV. DAILY FOOD INTAKE, GM.	FOOD INTAKE (% OF CONTROL PERIOD)
15	Control	7	317	
	200 cc. saline ¹	7	239	74.9
	200 cc. glucose 20% ²	7	273	86.1
16	Control	7	589	
	200 cc. saline	7	364	61.7
	200 cc. glucose 20%	7	650	110.3
17	Control	7	577	
	200 cc. saline	7	436	75.6
	200 cc. glucose 10% ³	7	569	98.6
16	Control	10	443	
	400 cc. glucose 20%	7	559	126.1
	400 cc. glucose 20%	9	409	92.3
17	Control	7	417	
	400 cc. glucose 20%	7	470	112.6
	400 cc. glucose 20%	9	427	102.4

¹ 0.85% NaCl. ² 20% Glucose in 0.3% NaCl. ³ 10% Glucose in 0.3% NaCl.

NaCl during one hour daily for from 7 to 9 days. As a control procedure, 3 dogs received equivalent volumes of 0.85 per cent NaCl intravenously during one hour daily for 7 days of the week preceding the glucose infusions.

The results indicate that glucose infusions failed to inhibit significantly food intake 20 minutes after the conclusion of the infusions. The percentage of food consumed ranged from 86.1 per cent to 110.3 per cent of the food eaten during the control period, and in no case did the decrement exceed that which occurred in the period of saline infusion.

In the second series of observation summarized in table 2, 5 dogs received from 584 to 960 cc. of 15 per cent and 1274 cc. of 10 per cent glucose in 0.3 per cent NaCl during 4 to 6 hours daily for periods of from 2 to 9 days. The volumes ranged from 146 cc/hr. to 212 cc/hr., depending on the weight of the dog. This represented a

rate of 1.7 grams of glucose/kg. of body weight/hour which is considered to be well within the capacity of the dog to remove glucose from the blood (7). Saline controls were performed as above.

TABLE 2

DOG NO.	PERIOD	NO. OF DAYS	AV. DAILY FOOD INTAKE, GR.	AV. VOL. DAILY CC.	% OF CONTROL CAL. INFUSED	% CHANGE FROM CONTROL PER.
15	Control	14	235			
	saline ¹	6	206	583		-12
	15% glucose ²	9	200	584	59.5	-15
16	Control	6	495			
	saline	2	377	923		-24
	15% glucose	3	446	960	43.8	-10
17	Control	14	391			
	saline	6	546	812		+39
	15% glucose	9	393	850	42.8	0
20	Control	6	366			
	saline	3	486	656		+32
	15% glucose	2	400	663	42.7	+9
19	Control	7	358			
	saline	7	302	1200		-15
	10% glucose ²	7	336	1214	110	-6

¹ 0.85% NaCl. ² 10% or 15% glucose in 0.3% NaCl.

TABLE 3

DOG. NO.	PROCEDURE	NO. OF DAYS	AV. DAILY FOOD INTAKE, GM.	% CHANGE FROM CONTROL PER.
15	Control	7	650	
	50% glucose ¹	2	705	+12
17	Control	7	1041	
	50% glucose	2	952	-8
18	Control	7	690	
	50% glucose	2	720	+4
19	Control	7	621	
	50% glucose	2	670	+9

¹ 20 cc. of 50% glucose.

The results indicate that the infusion in the form of glucose solutions of from 42 to 100 per cent of the calories eaten during the control period produced no significant corresponding depression of food intake. The decrement which ranged from 0 to

15 per cent was well within the spontaneous daily fluctuations of food intake seen in the control period, and in the periods of saline infusions, and was significantly less than the fraction of the daily caloric requirement infused.

In the third series, summarized in table 3, 4 dogs received 20 cc. of 50 per cent glucose intravenously on two separate days just prior to being fed. There was no significant depression of food intake as compared to the average daily food intake on control days. Although food was offered to the dogs for a period of 45 minutes, in almost all cases the dogs consumed their entire daily intake during the first 15 minutes and took no food during the remaining 30-minute period.

DISCUSSION

These results indicate clearly that intravenous glucose did not inhibit food intake in the dog under the conditions of these experiments. In considering the significance of these results two closely related aspects of the problem must be clearly distinguished. The first is the prompt effect on any specific meal of the intravenously administered glucose. The second is the effect of prolonged intravenous feeding on subsequent food intake.

As regards the first, the findings presented in this study indicate that elevating blood glucose levels by intravenous infusions of glucose in the experiments of series 1 and 3 had no inhibitory effect on food intake immediately after the infusions. Brobeck (8) has recently stated that "if there is indeed a central regulation of food intake, it must depend upon some reversible change which occurs during assimilation of food, a change able to activate or inhibit the sensitive cells." Our findings would indicate that supplying the organism with glucose, one of the products of normal digestion, does not effect such changes and cannot be considered responsible for the progressive inhibition of food intake when it results from the ingestion of a meal. This finding is also consistent with our recent observation that intravenously administered glucose in man is without effect on the hunger sensations which accompany deprivation of food (9). It is also in keeping with reports of Quigley and his coworkers that intravenously administered glucose did not inhibit gastric hunger contractions and intestinal motility in the dog (10, 11).

As regards the second aspect, mentioned above, the evidence presented in the experiments of series 2 is inconclusive, since only *dog 19* received seven consecutive daily infusions; the remaining 4 dogs received two or three consecutive daily infusions, alternating with periods of saline or control days. In this connection the fate of the extra calories given the dog is of importance since they may be concerned in the adjustment of food intake to bodily needs during longer periods of time than were studied in this experiment. Theoretically they may be stored as fat, expended as work, or dissipated as heat. During the short term of this experiment the weight of the dogs in series 2 remained relatively constant, varying by 0.1 to 0.2 kilograms. However the calculated weight gain, which would be expected if the glucose were stored as fat, would be small ranging from 0.08 to 0.5 kilograms (*dog 15*, 0.25 kg.; *dog 16*, 0.16 kg.; *dog 17*, 0.15 kg.; *dog 20*, 0.08 kg.; *dog 19*, 0.35 kg.) and except for the larger amounts would be detected with difficulty.

Increased activity may have occurred but was not noticed or measured in these

caged dogs. While increased metabolism, perhaps from the specific dynamic action of the materials infused, may be a third possibility, no evidence is available on this point. Controlled experiments on the effects on food intake of long continued feeding by other than oral routes are needed before this aspect of the regulation of food intake can be clarified.

SUMMARY

Daily intravenous administration of glucose had no significant inhibitory effect on food intake in the dog during periods of 2 to 9 days.

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LACTIC AND PYRUVIC ACIDS IN THE BLOOD AFTER GLUCOSE AND EXERCISE IN DIABETES MELLITUS^{1,2}

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THE characteristic relationships which normally exist between glucose, lactic acid and pyruvic acid have been described (1). This relationship is markedly altered in diabetes mellitus. Whereas the normal individual has a marked rise in lactic and pyruvic acid following the administration of glucose (2, 3), Bueding, Wortis and Fein (4) and Klein (5) have reported that the rise in the diabetic patient was either absent or delayed, and that insulin administration in diabetes increases the concentrations of lactic and pyruvic acid in the blood. Himwich (6) and the present authors² have shown that there is a dramatic rise of lactic and pyruvic acid in the diabetic subject after exercise. The present study is an attempt to provide a means of distinguishing mild diabetes from other forms of hyperglycemia and to learn more about the possible differences between carbohydrate metabolism at rest and during muscular work.

METHODS AND PROCEDURE

The glucose, lactic acid and pyruvic acid methods used have been previously described (1).

A group of known diabetics, both men and women, were selected in May 1944 for a study of the lactic and pyruvic acid changes in the blood which could be compared with those in subjects who had a B complex vitamin deficiency (7) and others who had an apparently normal carbohydrate metabolism. In the present continuation of these experiments not all of the former subjects were available and new ones had to be added. An asterisk in the tables indicates that the subject was available throughout the period of the study (1944-48).

All of the patients were deprived of insulin, either protamine zinc or regular, for at least 20 hours prior to the period of testing. The basal bloods were drawn in the early morning postabsorptive state after the patients had been completely relaxed for at least one-half hour. Due precautions were taken for the preservation of pyruvic acid, as suggested by Friedemann and Haugen (8). The amounts of glucose given orally was 1.8 gm/kg. of body weight. The tests described below are attempts to determine how the diabetic differs from the normal individual in his ability to change glucose to lactic acids with and without exercise. An extensive treatment of such data for non-diabetics is reported elsewhere (1, 7).

EXPERIMENTAL

Comparison of Normal and Hyperglycemic Conditions. For purposes of orientation, figure 1 contrasts the average glucose, lactic acid and pyruvic acid levels of 5 normal subjects and 5 diabetics, following glucose administration. The normal

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² Preliminary reports have appeared (*Federation Proc.* 5, 139, 1946, and Minutes of April 17, 1946, Meeting of Committee of Nutritional Aspects of Ageing, Food and Nutrition Board, National Research Council).

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curve shows a sharp rise in these three metabolites after glucose ingestion, returning to approximately basal levels in 180 minutes. Significant is the fact that the lactic and pyruvic acids rise and fall proportionately with the glucose. The diabetic curve, however, shows an early and prolonged elevation of glucose with almost no rise in lactic or pyruvic acid at 60 minutes and a slow rise thereafter. Some elevation of the levels of glucose, lactic acid and pyruvic acid still persists four hours after glucose administration. The lactate and pyruvate rise obtained in the diabetics is not greater than the normal rise but since the glucose increases are large the approximate 10 to 1 ratio of glucose to lactate is completely distorted.

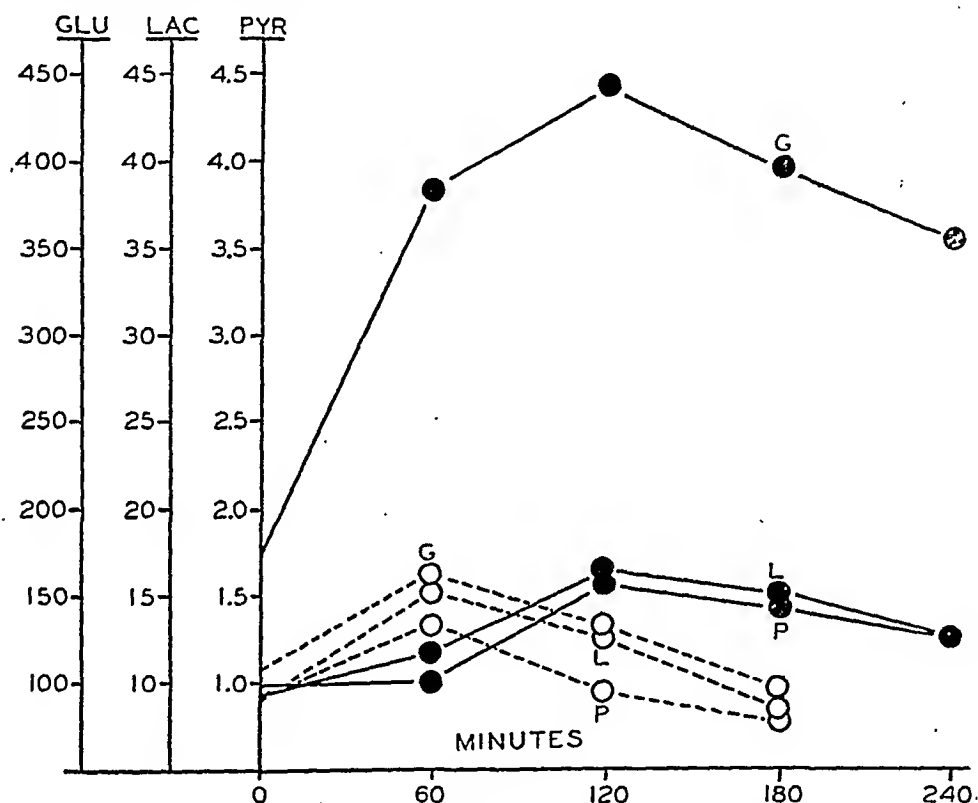


Fig. 1. COMPARISON OF NORMAL AND DIABETIC CURVES for blood glucose, lactic acid and pyruvic acid after glucose ingestion. Av. of 5 subjects in each group in mg. per cent. Interrupted line represents the normals; solid line, the diabetics. G, L, and P are glucose, lactic acid and pyruvic acid respectively.

This lag of the lactic acid and pyruvic acid levels following glucose administration affords a reliable test for the differentiation of true diabetes mellitus and of the prolonged equivocal glucose-time curves of other causes of hyperglycemia, either alimentary, circulatory or emotional. An example of such a test is given in figure 2 in which a subject, who is classified as an arteriosclerotic and who frequently has a high basal glucose and an elevated glucose-time curve, is compared with a typical diabetic. The arteriosclerotic subject, on whom the above observations were made in February 1944 (together with more than a dozen similar cases which have been frequently checked for a period of about 4 years), has not changed during the interim to give any indication of possible deterioration to a diabetic state.

Effects of Exercise with and without Glucose Ingestion. In order to evaluate the effect of exercise, both with and without glucose, in diabetes the standard exercise

test previously described (1) consisting of climbing and descending 21 ordinary steps twice in one minute, was applied to 7 diabetic patients and 6 non-diabetics. The

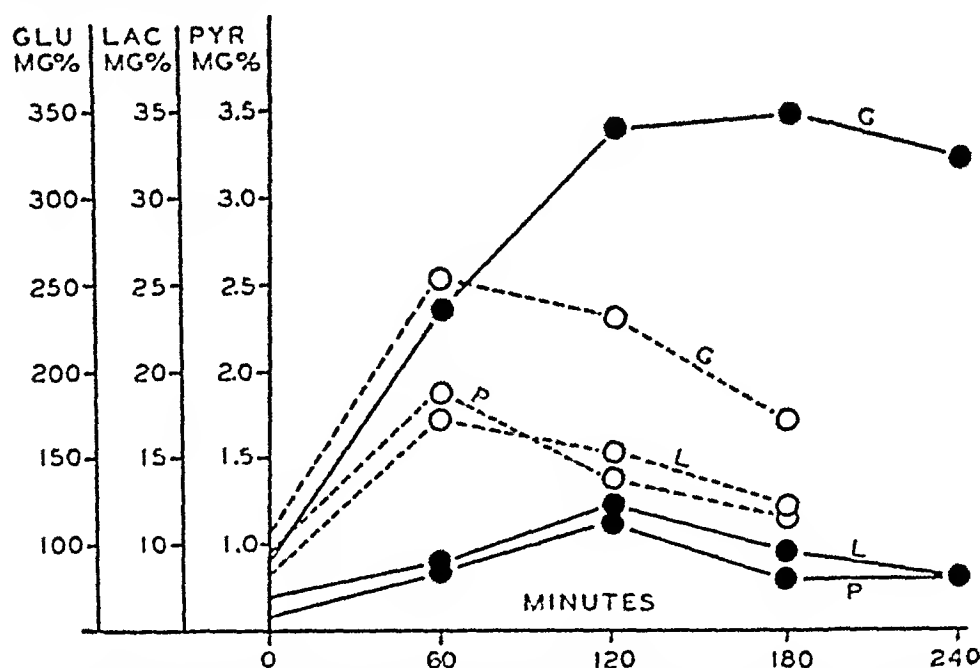


Fig. 2. COMPARISON OF GLUCOSE, LACTIC ACID AND PYRUVIC ACID LEVELS in an arteriosclerotic subject with a diabetic subject. Arteriosclerotic subject represented by interrupted lines.

TABLE 1. EFFECT OF MILD EXERCISE WITHOUT INSULIN OR GLUCOSE ON THE NORMAL AND DIABETIC SUBJECT

Age-sex			BASAL LEVEL MG. PER CENT			5 MINUTES AFTER EXERCISE			10 MINUTES AFTER EXERCISE		
			Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.
Non-diabetic											
J. S.	64	♂	114	13.1	1.16	122	16.4	1.39	124	13.2	1.50
E. R.	63	♂	114	10.5	.98	118	14.4	1.22	121	12.6	1.15
S. T.	77	♂	110	8.4	.80	116	11.6	1.08	118	10.2	.87
R. C.	27	♂	104	6.7	.90	110	15.7	1.30		9.7	1.02
J. L.	35	♂	99	6.8	.88	94		.94	94	8.0	.88
J. S.	20	♂	99	6.4	.74	102	10.4	.94	99	9.6	.94
Average.....			107	8.7	.91	110	13.7	1.15	111	10.6	1.06
Diabetic											
J. J.	67	♂	162	12.6	1.30	159	20.0	1.62	158	20.4	1.68
J. T.	62	♂	215	7.6	.70	213	17.6	1.30	208	16.0	1.32
M. E.*	59	♀	260	12.2	1.22	270	20.4	1.56	270	17.6	1.50
L. E.*	69	♀	240	11.8	1.14	238	20.2	1.66	237	15.5	1.36
R. S.	39	♀	232	11.2	1.06	230	22.4	1.58	240	15.8	1.36
J. B.*	51	♀	235	12.0	1.14	234	20.8	1.82	228	18.2	1.70
Average.....			224	11.2	1.09	224	20.2	1.59	224	17.3	1.49

results which include analyses of blood drawn after 16 hours of fasting and samples drawn 5 and 10 minutes after exercise are given in table 1. The data indicate that the blood glucose level is not affected by this amount of exercise but that there is a

definite rise in lactic and pyruvic acid which is approximately proportional to the blood glucose levels in both groups of subjects. The same mild exercise test conducted after the ingestion of glucose provided the data given in table 2. This exercise was given to 10 diabetics and 10 non-diabetics one hour after the standard glucose ingestion. Several facts are evident. The delayed rise of lactic and pyruvic acid in the diabetic 60 minutes after glucose administration is once again demonstrated, although the blood sugar levels increase from 60 to 100 per cent. This is contrasted with the rise in lactic and pyruvic acids in the non-diabetic. However,

TABLE 2. EFFECT OF EXERCISE PLUS GLUCOSE, WITHOUT INSULIN, ON THE NORMAL AND DIABETIC SUBJECT

Age-sex		BASAL LEVEL MG. PER CENT			60 MINUTES AFTER GLUCOSE			5 MINUTES AFTER EXERCISE		
		Glu.	Lac.	Pyr.	Gluc.	Lac.	Pyr.	Glu.	Lac.	Pyr.
Non-diabetic										
C. A.	36 ♂	110	7.4	.65	137	18.2	1.33	138	21.0	1.47
C. F.	32 ♂	95	12.5	.97	165	11.2	1.31	160	30.8	1.55
W. G.	38 ♂	110	7.8	.70	143	19.7	1.88	129	21.8	1.36
W. Gn.	41 ♂	108	7.7	.63	112	12.9	1.36	132	17.4	1.09
J. J.	46 ♂	89	8.4	.70	145	14.1	1.53	121	16.2	1.91
S. J.	29 ♂	115	7.7	.92	143	11.7	1.25	127	18.6	1.40
L. M.	38 ♂	85	5.5	.86	169	12.6	1.41	156	24.4	1.37
D. N.	29 ♂	104	9.2	.81	116	16.9	1.32	110	20.0	1.38
L. O.	42 ♂	108	7.9	.85	138	11.7	1.17	146	17.0	1.57
F. S.	29 ♂	111	7.4	.75	134	10.9	1.07	109	11.7	1.12
Average.....		104	8.2	.78	140	14.0	1.36	133	19.9	1.42
Diabetic										
J. J.	67 ♂	240	14.2	1.24	422	15.3	1.24	456	23.8	1.80
J. Mc.	81 ♂	195	12.3	1.06	368	16.5	1.52	344	36.4	2.60
J. T.	62 ♂	224	9.8	1.00	438	11.1	.90	458	22.6	1.80
J. R.	57 ♂	184	11.9	1.19	320	11.7	.94	364	26.2	1.40
T. H.	53 ♂	163	10.5	.98	318	11.4	1.08	372	23.8	1.60
R. S.	39 ♀	217	9.3	.92	422	9.5	1.00	442	23.8	1.78
M. E.*	59 ♀	334	18.9	1.62	600	22.0	1.46	672	34.8	2.84
L. E.*	69 ♀	334	14.7	1.20	448	16.5	1.20	490	43.5	2.42
S. S.*	57 ♀	340	21.4	2.24	486	22.6	1.94	522	38.4	2.58
J. B.	51 ♀	304	15.7	1.40	444	17.4	1.72	514	29.2	1.88
Average.....		254	13.9	1.29	427	15.4	1.30	463	30.3	2.07

here again as in table 1, there is a marked increase in the lactic and pyruvic acid levels in every case after exercise. This rise is higher in the presence of a high blood sugar than in the same patient exercised in the postabsorptive state.

These results show that the diabetic organism, which accumulates lactic and pyruvic acid very slowly in the blood in the resting state without insulin, can produce these metabolites promptly (5 min.) after exercise and that this rise is increased in the presence of either a very high blood sugar level or of exogenous glucose. This increase in lactic and pyruvic acid levels in the fasting diabetic, who has been exercised, is similar to that of the non-diabetic who has been given glucose plus exercise.

The non-diabetic, functioning at his basal blood sugar level, who climbs a flight of stairs shows a transitory mild rise of lactic and pyruvic acid. The uncontrolled diabetic has a basal blood sugar level which is equal to or higher than that of the non-diabetic who has ingested glucose. Therefore, since the blood lactic and pyruvic levels of the diabetic rise with exercise and, since this rise is greater when the blood sugar level is increased, one would expect the diabetic individual to have higher than normal lactic and pyruvic acid levels under average conditions of activity, even though resting figures may appear to be approximately within normal limits.

TABLE 3. EFFECT OF INSULIN ON GLUCOSE, LACTATE AND PYRUVATE AS RELATED TO VARYING TIME, DOSAGE AND EXERCISE

			BASAL (1 MIN. AFTER INSULIN)			30 MIN. AFTER INSULIN			36 MIN. AFTER INSULIN (5 MIN. AFTER EXERCISE)		
Age-sex		Insulin	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.
		u.	mg. %			mg. %			mg. %		
Diabetic											
J. J.	67 ♂	15	230	11.8	1.32	220	11.6	1.30	214	17.7	1.54
J. R.	57 ♂	10	140	14.3	1.24	149	12.7	1.08	144	16.3	1.30
J. T.	62 ♂	20	236	9.2	1.16	234	9.5	1.16	217	15.4	1.08
M. E.*	59 ♀	30	274	11.6	1.30	252	13.0	1.36	242	23.8	2.06
L. E.*	69 ♀	30	288	11.5	1.18	248	12.5	1.38	226	21.6	2.00
R. S.	39 ♀	20	246	7.3	.60	214	8.3	.72	211	17.1	1.16
J. B.*	51 ♀	20	288	13.2	1.24	248	13.3	1.44	241	27.8	2.06
Average.....			243	11.3	1.15	223	11.6	1.21	214	20.0	1.60

		30 MIN. AFTER INSULIN			60 MIN. AFTER INSULIN			90 MIN. AFTER INSULIN			96 MIN. (5 MIN. AFTER EXERCISE)		
Insulin		Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.
Diabetic													
J. J.	30	184	11.8	1.28	182	14.2	1.22	154	15.6	1.37	140	20.0	1.68
J. R.	20	152	10.6	.86	140	13.8	1.06	148	16.0	1.18	139	21.8	1.26
J. T.	40	246	9.9	1.00	260	14.0	1.42	237	21.6	1.08	229	23.5	1.12
M. E.*	60	294	11.5	1.38	270	21.8	1.76	246	21.8	1.83	240	26.7	1.84
L. E.*	60	296	15.4	1.30	252	17.8	1.90	234	18.0	1.80	211	36.5	2.14
R. S.	40	220	10.0	1.17	150	12.4	1.66	116	13.6	1.52	66	20.4	1.72
J. B.*	40	242	15.6	1.34	208	18.4	1.84	177	16.8	1.37	160	19.0	1.48
Average.....		233	12.1	1.19	209	16.0	1.55	187	17.6	1.45	169	24.0	1.61

Effect of Insulin on Glucose, Lactic Acid and Pyruvic Acid Levels. In order to learn whether the administration of insulin to diabetic patients produces a metabolic condition approximating the normal, two experiments were planned to distinguish between the effect of insulin alone and the effects of insulin plus glucose ingestion. In the first experiment (table 3) insulin was given immediately after basal blood samples were drawn and the glucose, lactate and pyruvate blood concentrations determined. Samples for analyses were also drawn 30 minutes later, and 5 minutes after the 1-minute stair climbing test. No change in lactate and pyruvate was noted 30 minutes after insulin injection and the response to exercise was similar to that previously obtained without insulin. Since no response was obtained in 30 minutes

with the dose of insulin used and, since it is important to evaluate the question discussed by Klein (5) regarding the ability of insulin to raise lactic and pyruvic acid levels without the addition of glucose, another experiment was conducted. In this case the insulin dose was doubled on the same patients and the exercise was not applied until 90 minutes after the insulin injections. Again no conclusive change was noted in 30 minutes but a definite rise was obtained at 60 and 90 minutes, accompanied by a fall in blood sugar levels. This is definite evidence that insulin per se in the diabetic will cause an increase in the lactic and pyruvic acid blood levels. The response to exercise appears the same in the presence of insulin as without insulin (compare table 1).

Another series of experiments (table 4) studied the combined effects of insulin and glucose ingestion. When insulin was given one-half hour after glucose, there was a definite rise of the lactic and pyruvic acid levels within 30 minutes. This response indicates that in the presence of insulin the lactate and pyruvate rise following glucose ingestion in the diabetic subject is accelerated (compare with fig. 1 and table 3). When insulin was given simultaneously with the glucose, a similar response was obtained. The effect of insulin action on the levels of lactic and pyruvic acid following exercise and glucose was not different from the postexercise effects obtained with glucose but without insulin (compare with table 2).

DISCUSSION

The demonstration that the diabetic patient has little if any increase in blood lactic and pyruvic acid during the first hour following glucose ingestion confirms the report of Bueding, Wortis and Fein (4) who studied pyruvate changes. The delayed rise in lactic and pyruvic acid described by Klein (5) was also observed in our experiments. This rise is not less than that obtained with non-diabetic subjects with average glucose tolerance curves (fig. 1) except that the peak is obtained in the vicinity of 120 minutes instead of 60 minutes after glucose ingestion. The main difference in lactate and pyruvate response between diabetic and non-diabetic subjects is in the ratio between the glucose and the lactate or pyruvate concentrations obtained. The high glucose-time curves seen in alimentary hyperglycemia (1), or cardiovascular disorders, are accompanied by proportionately larger increases in lactate and pyruvate and are therefore distinguishable from the curves of the resting diabetic in whom the lactic and pyruvic acid concentrations never approach the relative heights of the glucose.

The ability of the diabetic to convert carbohydrate to lactic and pyruvic acid by muscular work is apparently unimpaired. The high lactic and pyruvic acid levels obtained after exercise are as high as those which would be realized if a non-diabetic with a very high blood sugar were given the same mild exercise (1, 7). Since a moderate degree of activity, such as the stair climbing test, is a part of normal living, it is clear that the uncontrolled diabetic is functioning for a large proportion of his waking hours with abnormally high levels of lactic and pyruvic acid in the blood. Even many controlled diabetics who have been adjusted to blood sugar levels of 160 to 200 mg. per cent would be expected to have prolonged abnormal levels of lactate and pyruvate, especially following mild exertion after meals. Whether or not these high levels have anything to do with the pathogenesis of the neurological and vascular

lesions found in diabetes mellitus is not known but they should be further evaluated in making future decisions on the management of the diabetic. Though the high levels of lactate and pyruvate found in thiamine deficiency have been correlated with the neurological changes which occur (1, 7), no direct cause and effect relationships have, as yet, been proved.

Interpretation of the data in this paper is simplified if one accepts the hypoth-

TABLE 4. EFFECT OF INSULIN AND GLUCOSE INGESTION ON BLOOD GLUCOSE, LACTIC AND PYRUVIC ACIDS BEFORE AND AFTER EXERCISE

			BASAL LEVELS MG. PER CENT			60 MIN. AFTER GLUCOSE			66 MIN. AFTER GLUCOSE (5 MIN. AFTER EXERCISE)		
Age-sex		Insulin units at 30 min. after glu. ingestion	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.	Glu.	Lac.	Pyr.
Diabetic											
J. J.	67 ♂	15	262	13.0	1.12	426	13.7	1.28	460	20.6	1.54
J. M.	81 ♂	10	208	11.9	1.16	400	17.7	1.44	398	30.3	1.78
J. T.	62 ♂	20	240	9.2	1.04	388	12.6	1.18	370	20.2	1.62
J. R.	57 ♂	10	190	12.9	1.00	318	13.2	1.08	346	25.8	1.36
T. H.	53 ♂	10	182	8.9	1.10	360	12.9	1.36	356	23.1	1.66
R. S.	39 ♀	20	232	7.5	.90	408	12.9	1.10	394	21.6	1.44
M. E.*	59 ♀	30	298	15.1	1.18	522	26.0	1.74	518	41.0	2.62
L. E.*	69 ♀	30	320	17.7	1.32	440	23.0	1.60	440	40.0	2.10
S. S.*	57 ♀	20	244	19.0	1.32	372	22.5	1.54	376	42.5	2.28
J. B.*	51 ♀	25	280	12.4	1.18	470	19.6	1.54	460	23.1	1.52
Average.....			246	12.8	1.13	410	17.4	1.39	412	28.8	1.79
		Insulin with glucose									
J. J.		15	288	11.2	1.22	402	16.0	1.60	400	21.6	1.84
J. M.		10	188	9.9	1.00	324	17.0	1.85	340	36.6	2.54
J. T.		20	248	11.7	1.30	308	15.4	1.40	308	24.0	1.74
J. R.		10	176	7.7	.98	296	9.9	1.00	300	23.4	1.42
T. H.		10	182	11.8	1.34	308	16.5	1.82	314	31.2	2.54
R. S.		20	216	8.7	.80	344	11.7	.95	336	22.5	1.76
M. E.*		30	304	15.6	1.40				540	39.0	2.78
L. E.*		30	328	16.6	1.30	416	21.1	1.74	406	48.4	2.54
S. S.*		20	268	10.8	.88	328	22.2	2.00	332	45.3	2.40
J. B.*		25	316	16.5	1.40	492	18.9	1.44	442	28.0	1.78
Average.....			251	12.0	1.16	358	16.5	1.53	372	32.0	2.13

esis that insulin is not required in the breakdown of carbohydrate for muscle work. The need for insulin in the glucose \leftrightarrow liver glycogen system which is active even when the individual is at rest is currently an accepted fact with which these results do not conflict. The separate consideration of carbohydrate metabolism data, obtained when the organism is at rest from those obtained during muscular work, might help to clarify conflicting reports on the mode of insulin action. Comparison of glucose to lactic and pyruvic acid reactions obtained in the mammal with those ob-

tained in *in vitro* experiments are best postponed until there is clarification of the variations reported with different tissue preparations (9).

SUMMARY

The effect of glucose ingestion on levels of blood lactic and pyruvic acid in diabetic and non-diabetic subjects has been compared. The diabetic has a delayed rise in the levels of lactic acid and pyruvic acid. Comparison of alimentary hyperglycemia with diabetes mellitus indicates that the resting non-diabetic has a more rapid lactate and pyruvate response to glucose ingestion than the resting diabetic, who does not accumulate as much lactate and pyruvate in the blood even though his glucose load is much higher than subjects with alimentary hyperglycemia. This difference is especially large during the first hour after glucose ingestion since the diabetic exhibits a marked delay in the rise in lactic and pyruvic acids which normally occurs following glucose ingestion. The use of this fact provides a possible means of distinguishing between functional hyperglycemia and true diabetes mellitus.

The lactate and pyruvate response to exercise is apparently normal in the diabetic. The prompt rise obtained when carbohydrate is utilized during muscular work contrasts with the lag obtained when glucose is ingested in the absence of exercise. Since high glucose levels increase the response to exercise, the uncontrolled diabetic shows proportionately higher levels of lactic and pyruvic acids after mild exertion.

The lactate and pyruvate response to exercise is not altered by the action of insulin. Insulin injection in the fasting diabetic has little if any effect on lactate and pyruvate levels in 30 minutes but causes a rise in 60 minutes. Insulin in conjunction with glucose ingestion shows greater apparent activity, as indicated by an increase of lactate and pyruvate in 30 minutes after insulin despite the lower glucose level.

Since the average diabetic is maintained at a higher than normal blood glucose level and since his normal activities includes exertions which are greater than the mild exercise test herein described, it may be concluded that many diabetics exist, during many of their waking hours with pathological high levels of lactic and pyruvic acids.

The data presented are consistent with the concept that insulin is involved in the conversion of glucose to lactic and pyruvic acids, but only when the individual is at rest. The amounts of lactic and pyruvic acids found in the blood after mild exercise are, as in the non-diabetic, proportional to the blood glucose, so that one may conclude that insulin is not necessary to convert carbohydrate to lactate and pyruvate during exercise.

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INCIDENCE, CONTROL AND REGRESSION OF DIABETIC SYMPTOMS IN THE ALLOXAN-TREATED RAT

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IN A pilot experiment in this laboratory, a spontaneous regression in the severity of diabetes as measured by the amount of glucose excreted daily in the urine has been observed in each of 10 rats rendered diabetic by alloxan, and maintained thereafter on a fixed amount of a stock diet. At the end of the fifth week after alloxan injection, carbohydrate retention had returned to normal in half of the survivors, and in the other half had fallen to an equilibrium value. A renewed excretion of urinary glucose was produced and observed for 12 weeks in 4 of the completely recovered animals by the administration of a second similar dose of alloxan. In this paper we propose to describe these results very briefly, and those of other experiments designed to study the relation of insulin to this type of diabetes.

EXPERIMENT A

Materials and Methods. Seventeen female Wistar rats, having uniform initial body weights (176 ± 22 gm.), received 200 mg/kg. of alloxan monohydrate¹ in a single subcutaneous injection following a 48-hour fast, according to the method of Kass and Waisbren (2). The animals were then placed in individual screen-bottomed metabolism cages set over large waxed funnels permitting quantitative collection of urine under toluene. Samples were removed daily and stored at refrigerator temperature in Erlenmeyer flasks. The total volume of urine and rinse water collected per week from each rat was then measured and the concentration of reducing substances determined quantitatively by a modification of the Miller-Van Slyke method for blood sugar (3). The reducing substances were shown by periodic yeast fermentation tests to be over 99 per cent glucose; therefore the rate of excretion of these substances is expressed as grams of glucose per week.

Throughout the entire period of observation, each animal was offered 14.0 grams of ground Purina Fox Chow² each day in an anchored food bin. As this procedure was found in preliminary trials to result in negligible scatter, the amount consumed per day was taken as the difference between the amount offered and the residue. The food offered was usually entirely consumed.

Initially the pancreases were fixed by intravascular perfusion of the anesthetized animal with Helly's fluid (Zenker solution with 5% neutral formalin in place of glacial acetic acid). More consistent results were obtained by immersing the tissues for 48 hours in Bouin's solution prepared with 3 per cent trichloroacetic acid in place of the usually employed glacial acetic.³ This latter method was used for the majority of the tissues. Dehydration and clearing were carried out in 10 changes of dioxane over a period of 16 hours.⁴ Infiltration with tissue-mat⁵ was effected in a vacuum oven (20

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¹ The alloxan preparation used in this series of experiments was obtained from the Eastman Kodak Company, Rochester, N. Y. It was found to be pure alloxan on the basis of the titrimetric method of Archibald (1).

² According to a statement obtained from the manufacturers, this feed contains 54% carbohydrate, 4% fat and 20% protein.

³ Suggested by Prof. L. C. Simard, University of Montreal, Montreal, Canada.

⁴ Using the Autotechnicon, Technicon Company, New York City.

⁵ Fisher Scientific Company.

inches of water below atmospheric pressure) at 58°C., passing the tissues through five changes over a period of 8 hours. Three micra sections were routinely cut, taking care not to allow them to float on water sufficiently long while mounting that the yellow color due to the picric acid content faded. Satisfactory differential staining of the islet cells could not be obtained if the sections did not still retain the yellow color before staining. Haematoxylin and eosin, azocarmine, anilin-blue and orange-G, and Bowie's stains were performed on sections from each block. In the course of this latter staining procedure it was found absolutely essential that the section be blotted as uniformly dry as possible. Otherwise the removal of any water remaining in the section by absolute alcohol or acetone resulted in the simultaneous removal of too much stain, frequently in an irregular fashion. Gomori's chromium haematoxylin and phloxine method was also employed in selected cases. This combination of stains not only enabled the alpha cells to be sharply differentiated from the beta cells, but also made possible the estimation of the approximate number of granules present in the latter.

Results. Under the fixed conditions described above, a sustained decrease in the severity of the diabetes, as measured by the quantity of glucose excreted per week in the urine, was observed in each of the surviving diabetic animals (table 1). This improvement was most rapid during the first weeks after alloxan, and resulted in an almost complete disappearance of glucose from the urine in 5 (29%) of the rats, and in a less pronounced regression in another 5. Of the remaining animals, one (6%) died within the first week after alloxan, and the remaining 6 (36%) exhibited little or no glycosuria. This last group of animals was not studied beyond the second week.

The fasting blood sugar levels of the 5 rats which remained glycosuric 10 to 12 weeks after the alloxan treatment (nos. 4, 5, 7, 10 and 13) were determined after a one-day fast, after which they were anesthetized with sodium amytal and their pancreases removed (4). The pancreases of 2 of these rats (nos. 10 and 13) were fixed for histological examination, while those of the remaining 3 were pooled and extracted by the method of Scott and Fisher (5) for estimation of their insulin content, using a mouse convulsion method (4).

The average fasting blood sugar level in these animals at time of death was significantly higher than the average for fasting normal female rats of the same weight and under similar treatment (195 ± 24 mg. % as compared with 84 ± 2 mg. %), confirming the conclusion, based on measurements of urinary glucose excretion, that these animals were diabetic. The average insulin content of pancreas of the 3 rats in this group was found to be 0.14 U per rat, a value less than 5 per cent of that observed in this laboratory in normal rats of the same sex and equivalent weight maintained on the same ration and killed after a 24-hour fast.

With the haematoxylin and eosin stain neither islet nor acinar tissue showed obvious deviations from normal in either of the 2 animals examined, except that islet tissue was difficult to find. No increase in connective tissue within the islets was seen, using the Masson stain. Cells near the periphery of such islets were identified as alpha cells, using both Masson and Bowie stains, and on comparison with normal animals the alloxan diabetic animals showed a distinct increase in the proportion of alpha cells to the total number of islet cells. Centrally located cells in these islets stained poorly as compared with the response of similarly located (beta) cells in the islets of normal rat pancreas.

The 5 rats which had shown a transient glycosuria were each given a second subcutaneous dose of alloxan, equal in magnitude to the first, at 9 to 11 weeks after the

TABLE 1. TOTAL GLUCOSE EXCRETED/RAT/WEEK AFTER FIRST AND SECOND DOSES OF ALLOXAN (200 MG/KG.) (EXPERIMENT A)

RAT	Dose: 14.0 gm. Purina Fox Chow/day																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	10.9	3.9	3.9	2.4	1.1	1.5	0.6	1.2	0.9	0.6	0.9 ¹	8.4	11.0	6.4	22.1	14.7	24.5	31.0	15.3
2	5.8	3.1	3.1	1.4	0.7	1.0	0.5	0.7	0.7	0.5	0.3 ¹	0.4	3.9	7.7	13.9	15.6	11.9	31.9	15.4
14	23.2	15.2	9.4	5.3	1.3	1.3	2.0	1.2	0.3 ¹	0.3	Dead								
15	17.2	9.7	4.1	3.0	2.4	2.2	1.8	1.8	0.8 ¹	3.2	2.1	1.3	8.3	7.3	5.7	1.9	13.6	7.2	7.4
16	10.0	2.2	0.2	0.2	0.2	0.1	1.2	0.5	0.1 ¹	9.9	17.8	15.4	21.8	19.4	Dead				8.3
4	19.0	10.6	8.1	7.6	5.3	4.0	7.2	3.8	6.4	6.7	7.0 ²								
5	17.9	11.7	5.9	5.5	7.6	6.2	6.4	5.2	4.4	5.9	4.0 ²								
7	15.4	16.4	6.5	8.8	8.3	8.4	6.9	4.5	4.9	5.9	5.0 ²								
10	22.4	16.3	9.8	8.4	5.4	5.8	5.1	4.2	5.6	6.0	4.5 ²								
13	20.0	16.3	13.3	11.7	9.5	10.8	9.8	10.4	7.1	8.7 ²									

¹ These animals received a second dose (200 mg/kg.) of alloxan at the end of the week indicated.² These animals were killed for insulin assay and histological study of pancreas at the end of the week indicated.

initial one. The weekly excretion of glucose in the urine was followed for each animal through an additional period of 12 weeks, or until the death of the animal. Throughout this time the same ration was provided as during the period of observation following the first dose of alloxan. In each of these animals, except no. 14, which died shortly after the second alloxan treatment, there was a return of sustained and pronounced glycosuria within the period of observation (table 1), indicating that the second dose of alloxan had again been effective in reducing the ability of these animals to metabolize carbohydrate in a normal fashion.

EXPERIMENT B

Experiment B represents an extension of observations already published (6) concerning the changes in insulin content of rat pancreas during the first two days after alloxan, and observations on the effect of exogenous insulin on the excretion of glucose in the urine by rats with severe and sustained alloxan diabetes.

Materials and Methods. Seventy-four mature Wistar rats of both sexes were sorted into 8 groups, each having approximately the same initial weight distribution and average weight, 240 grams/rat. Animals in five of the groups received subcutaneous injections of 350 mg/kg. of alloxan distributed between four equal doses, administered at 15-minute intervals following a 24-hour fast. The remaining groups, serving as controls, received no alloxan. All groups killed within the 48 hours following administration of the alloxan were fasted during the period of their survival.

Groups of rats were totally depancreatized under sodium amytal anesthesia at the times indicated in table 2. The pancreas of each animal in a given group was deposited immediately after removal in a single container of acid alcohol, and the insulin determined as in *Experiment A*.

One group of the alloxan-treated animals and one of the normal controls were allowed to survive through a period of 72 days after alloxan injection. These rats were kept in individual metabolism cages, the normal controls being fed on the following day the same average amount of ground Purina Fox Chow as that which the alloxan-treated group consumed on a given day. Pancreases were removed for insulin content determinations following the procedure used in the earlier studies.

In order to determine the action of insulin on the excretion of urinary glucose in the alloxan-diabetic rats, a group of 4 adult Wistar rats, selected because of the severity of their alloxan diabetes of approximately two months' standing, were maintained in individual metabolism cages equipped to permit collection of 24-hour urine samples. Each animal was offered a ration of 13.0 gm. of ground Purina Fox Chow per day. A standard dose of 0.80 \bar{u} of protamine zinc insulin was administered subcutaneously from a micrometer syringe to each rat once a day through seven-day periods. These periods were alternated with ones of equal length during which the dose was reduced to 0.40 \bar{u} , but with other treatment remaining the same. The total excretion of reducing substances in the urine was measured daily for each rat over a period of several weeks.

Results. The observations illustrated in figure 1⁶ demonstrate that within a period of 48 hours following the administration of alloxan the insulin content of pancreas falls progressively to a very low level (4.3 % of normal in the present case). That this fall is largely a result of the action of alloxan is confirmed by a comparison of the insulin contents of the alloxan-treated and control groups at 37.5 and 38.5 hours, respectively, after the time of alloxan administration. While the insulin content of pancreas in the alloxan-treated group 72 days after alloxan administration is still well below that of the control group (table 2), it is more than four times as

⁶ The results shown in fig. 1 include previously published figures (6) obtained from a group of similar animals subjected to the same treatment. We are grateful to Dr. J. H. Ridout for her continued collaboration during the course of this experiment.

large as that observed in the 48-hour group, suggesting that some increase in insulin content of pancreas may have occurred between the 2d and 72d day after alloxan.

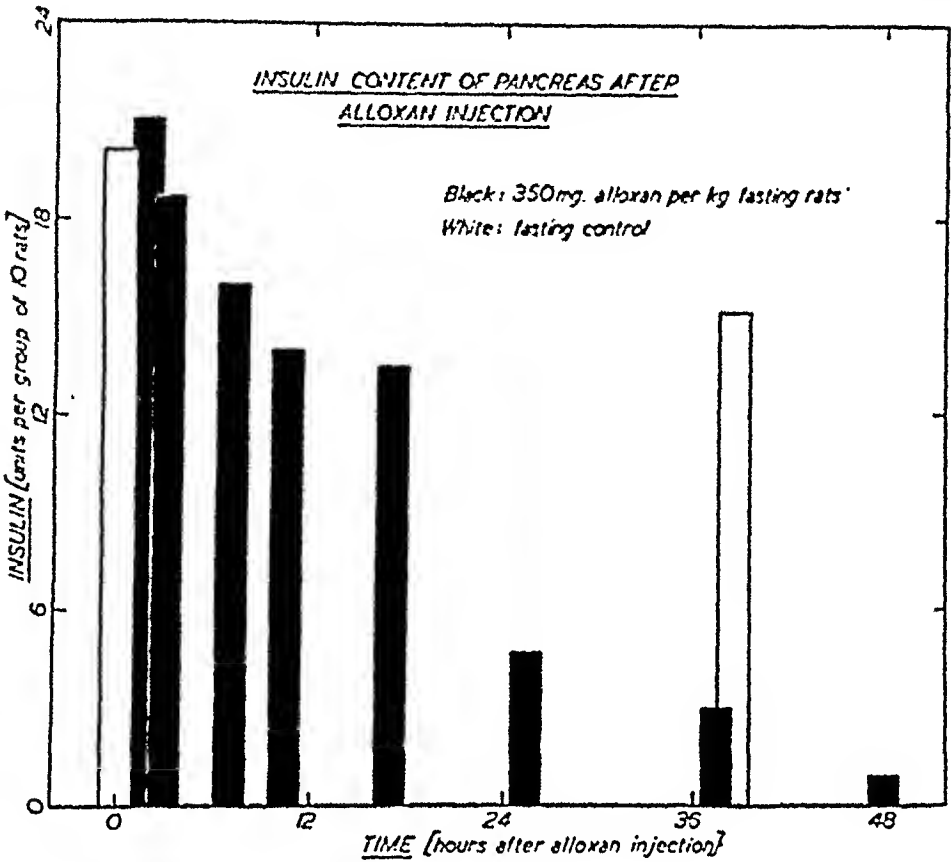


Fig. 1

TABLE 2. INSULIN CONTENT OF RAT PANCREAS AS A FUNCTION OF TIME AFTER SUBCUTANEOUS INJECTION OF 350 MG/KG. OF ALLOXAN (EXPERIMENT B)

TREATMENT	NO. OF RATS IN GROUP	TIME AFTER ALLOXAN	INSULIN (U/GROUP OF 10 RATS)	NO. OF MICE USED IN ASSAY
Control.....	6	0.0 hours	19.0	132
Alloxan.....	7	10.7 "	14.0	132
Alloxan.....	9	17.0 "	13.5	132
Alloxan.....	12	25.5 "	4.7	120
Alloxan.....	13	37.5 "	3.5	120
Control.....	6	38.5 "	15.1	120
Control.....	5	72 days	9.6	132
Alloxan.....	16	72 "	3.9	167

The effect of insulin on the excretion of glucose in the urine of the 4 rats with severe alloxan diabetes of several weeks' duration is illustrated in figure 2. It is seen that those periods in which the daily dose of insulin is increased are in every case associated with periods of decreased excretion of glucose in the urine, and vice versa, with a one-day phase lag between therapy and measured response.

Throughout the period of treatment with insulin these rats were voracious, often consuming their entire day's ration as soon as it was offered. Daily food consumption records show that the dietary intake of these animals was the same in periods of reduced insulin injection as in those where the full dose was given.

EXPERIMENT C

This experiment was designed to observe concurrent long-term changes in body weight, urinary glucose excretion, fasting blood sugar levels and the histology and insulin content of pancreas in strictly comparable groups of rats treated with alloxan and subsequently rigidly maintained on a fixed diet.

Materials and Methods. Seventy-two mature female Wistar rats having a restricted range of body weights (197 ± 7 gm.) were divided into groups which were made comparable on a basis of weight distribution. These groups received the treatments outlined in table 3. A dose of 200 mg/kg. of alloxan was administered subcutaneously to all but the control animals following a 48-hour fast. The survivors were placed in individual metabolism cages and were provided with a daily ration of 14.0 gm. of ground Purina Fox Chow per animal.

At time of death an anesthetic dose of sodium amytal was administered and the abdomen and thorax opened. A sample of cardiac blood was removed to determine blood sugar concentration, and the animal was then totally depancreatized. The pancreas was dropped immediately into a container of acid alcohol, one for each group of rats, and minced with scissors as a first step in insulin extraction. The pancreases of two representative⁷ animals from each group were fixed in Zenker-formol solution for histological examination.

Body weights and total glucose excreted in the urine were measured each week for surviving groups, the rats in each control group being fed the same average weight of ration as was consumed the day before by the rats in the corresponding experimental group. The entire ration offered each day was consumed after the first week following alloxan injection (figure 3). During the seventh and eighth weeks of the experiment the normal animals of control *Group IVa* were maintained in metabolism cages, and their total weekly urine samples collected and analyzed quantitatively for their content of reducing substances. When expressed as grams of glucose per week, these values were found to be effectively constant, and the same in magnitude for different rats in the group. They were found to represent approximately 0.77 ± 0.13 per cent of the total reducing substances excreted in the urine of the alloxan diabetic rats in *Group IVb* during the same period.

Since some of the estimations of insulin content of pancreas were made on small groups in which the amount was known to be low, it was found necessary to devise a semi-micro method to obtain this information. The fall in blood sugar concentration in fasting mice following injection with insulin was used as an index. The Miller-Van Slyke micro method of blood sugar measurement (3) was employed, 0.10 cc. of blood being withdrawn into a dried heparinized pipette from the tip of the freshly-sectioned tail of each mouse one hour after subcutaneous injection of 0.25 cc. of a solution of an unknown insulin made up in isotonic acid saline (pH 2.5). The group-average blood sugar concentration was compared with that of a similar group of mice each injected with an equal volume of similarly prepared standard insulin of commensurate concentration, and with a third group injected with the acid saline alone. The potencies of the insulin solutions were measured in terms of the differences in blood sugar level existing between the average for the saline group and those for the

⁷ Animals whose pre-existing urinary glucose excretion corresponded approximately with the group average were selected as being representative specimens for histological study.

insulin-treated groups, each referred to a separately-determined curve for mice relating blood sugar response to insulin dosage. A week after such a test a second one was performed in which the groups which had received the unknown and standard insulins were interchanged. In all groups for which a sufficient supply of extracted insulin was available, assays were made by the mouse convulsion method, as well as by the above procedure. The insulin concentrations obtained by the two methods of assay were found to differ by less than 16 per cent in four different solutions tested.

In order to provide a biometrical basis for assessment of the significance of differences in insulin content of pancreas determinations as measured in *Experiment C*, a supplementary experiment was made in which 50 female Wistar rats of 164 ± 13 gm. initial body weight per rat were subdivided into five groups of 10 rats each. Four of these groups were injected subcutaneously with 200 mg/kg. of alloxan after a 48-hour fast. These groups were depancreatized 48 hours later, following the

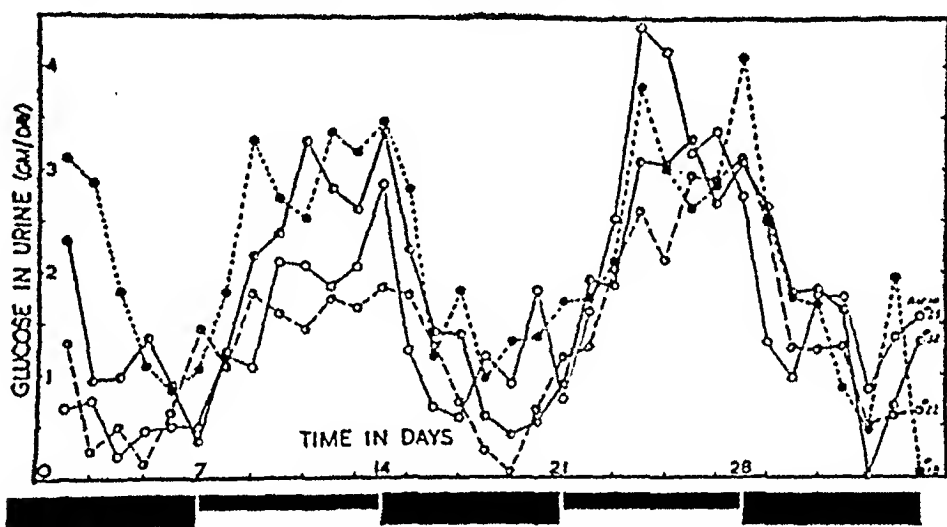


Fig. 2. EFFECT OF PROTAMINE-ZINC INSULIN on glycosuria of alloxan-diabetic rats on fixed diet (*Experiment B*). Alternating levels of insulin dose are shown in black beneath the time axis.

same treatment and procedure as used in *Experiment C*, and the insulin content of pancreas determined by groups using the mouse methods of assay referred to above. It is seen that the average insulin content of pancreas, when measured under the conditions described, amounts to 0.63 U/group of 10 rats with a standard deviation of 0.28 U/10 rats. The small amount of insulin present in this group of pancreases made the measurement of insulin content more difficult than for any other attempted. For this reason the standard deviation for other groups containing the same number of animals is undoubtedly less than that observed here. When compared with the value of 12.0 U/group of 10 rats in the normal controls, it is seen that the value 48 hours after alloxan represents 5.3 ± 1.4 per cent of normal.

Results. Average trends in metabolic factors for the long-term subgroups of animals in *Experiment C* are illustrated in figure 3. Statistically significant differences in trends of glucose excretion with time are consistently observed after alloxan injection in Groups *IIIb*, *IIIc*, and *IVb*, justifying the procedure used in subdividing the alloxanized animals into these groups.

These trends correspond with those reported in *Experiment A* for alloxan-treated rats maintained under similarly controlled conditions. In both experiments the animals surviving for more than one week showed either a sustained and pronounced glycosuria, a transient glycosuria, or were aglycosuric. The number of surviving alloxan-treated animals falling into each of these classifications in *Experiment C* is shown in table 3. The proportion of animals falling in each subdivision is similar

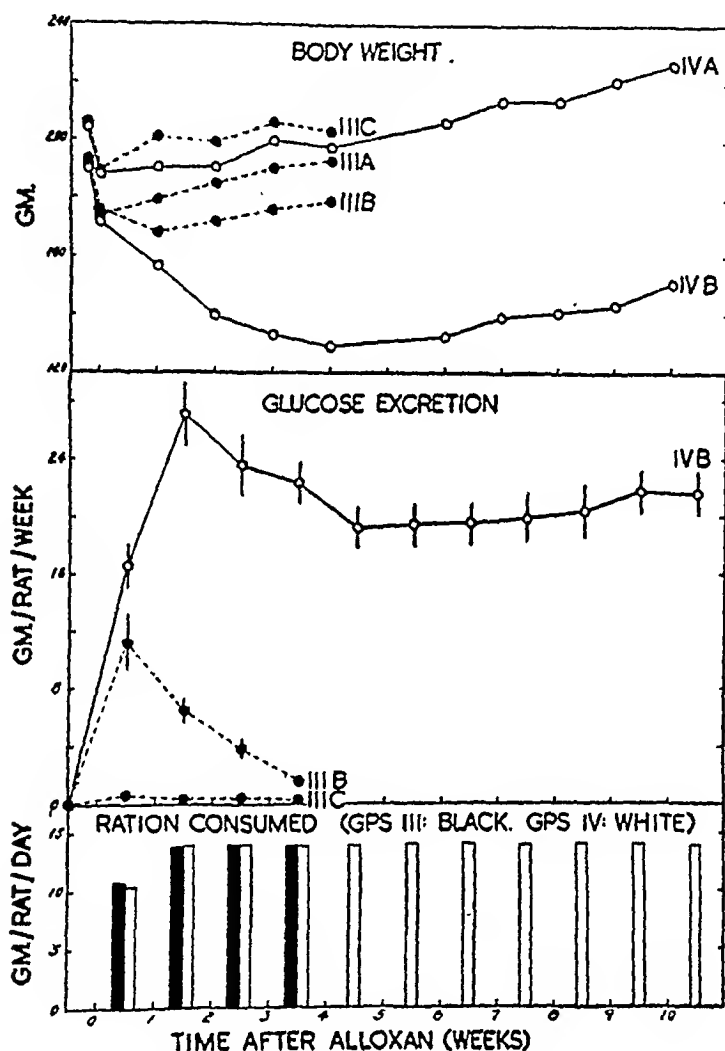


Fig. 3. LONG-TERM TRENDS in body weight and rate of glucose excretion compared with dietary intake in groups of alloxan-treated and control rats (*Experiment C*).

to that described earlier for *Experiment A*. In both experiments there was a progressive improvement in the carbohydrate retention of *all* glycosuric animals during several weeks, a condition of relatively constant glycosuria or of aglycosuria being approached asymptotically in most cases. The amount of glucose excreted per rat per week in the days immediately following alloxan administration is larger in the groups which showed sustained glycosuria than in the corresponding groups with transient glycosuria.

The weekly consumption of dry ration in *Experiment C* was commensurate at all times for all groups (figure 3). Hence changes in body weight should be related

to the corresponding values of urinary glucose excretion. That such relations exist is supported not only by the effectively constant and equal gains of weight by the

TABLE 3. SUBDIVISION AND TREATMENT OF RATS (EXPERIMENT C)
200 mg/Kg. of alloxan were administered to the groups indicated on zero day.

GROUP NO.	TOTAL ANIMALS IN GROUP	TREATMENT OF ANIMALS	SURVIVAL TIME AFTER ALLOXAN DAYS	HRS. OF FAST BEFORE PANCREATICTOMY
<i>Ia</i>	8	Normal controls	0	48
<i>IIa</i>	10	Normal controls	2	24
<i>IIb</i>	11	Alloxan-treated	2	24
<i>IIIa</i>	8	Normal controls	30	24
<i>IIIb*</i>	4	Alloxan-treated. Transient glycosuria	30	24
<i>IIIc</i>	6	Alloxan-treated. No glycosuria	30	24
<i>IVa</i>	8	Normal controls	72	24
<i>IVb*</i>	10	Alloxan-treated. Sustained glycosuria	72	24

The division of the alloxan-treated animals among the groups marked with an asterisk was based on the pattern of change in urinary glucose excretion followed in each animal during the first 4 weeks of observation.

TABLE 4. CORRESPONDING VALUES OF FASTING BLOOD SUGAR LEVELS AND INSULIN CONTENT OF PANCREAS IN RATS (EXPERIMENT C)

GROUP NO.	NO. OF RATS IN GROUP FROM WHICH INSULIN WAS EXTRACTED	DAYS AFTER ALLOXAN WHEN KILLED	INSULIN/GROUP OF 10 RATS		FASTING BLD. SUG. \pm S.E. MG. %
			U	% of control group value	
<i>Ia</i>	8	0	10.7	100.0	81 \pm 2
<i>IIa</i>	8	2	11.7	100.0	88 \pm 3
<i>IIb*</i>	9	2	0.32	2.7	352 \pm 35
<i>IIIa</i>	4	30	11.5	100.0	86 \pm 4
<i>IIIb*</i>	2	30	2.28	19.8	108 \pm 7
<i>IIIc</i>	4	30	9.0	78.3	102 \pm 3
<i>IVa</i>	6	72	19.0	100.0	80 \pm 3
<i>IVb</i>	8	72	1.5	7.9	263 \pm 37

Groups marked with an asterisk are those in which insulin content of pancreas was determined by the mouse blood sugar method alone. In most of the other groups 200 mice were used in mouse convulsion assays of each unknown.

aglycosuric and control groups of animals, but in particular by the relation between glucose excretion and change in body weight of *Group IVb* animals relative to their controls of *Group IVa*.

The insulin content of pancreas and fasting blood sugar values for the various groups described in table 3 are presented in table 4. A pronounced difference between the insulin content of pancreas of experimental and control groups is found to exist in every case where the fasting blood sugar of the experimental animals is elevated, or where there has been even a transient glycosuria. In some cases the standard dose of alloxan used produced no signs of diabetes (*Group IIIc*), but there was histological evidence of lasting damage to the islets of Langerhans and a subnormal insulin content of pancreas 30 days after alloxan administration.

In normal control animals a peripheral shell of alpha cells was found nearly to encompass a larger volume of beta cells (fig. 5A). Forty-eight hours after alloxan administration the only islet tissue which appeared to be intact consisted of alpha

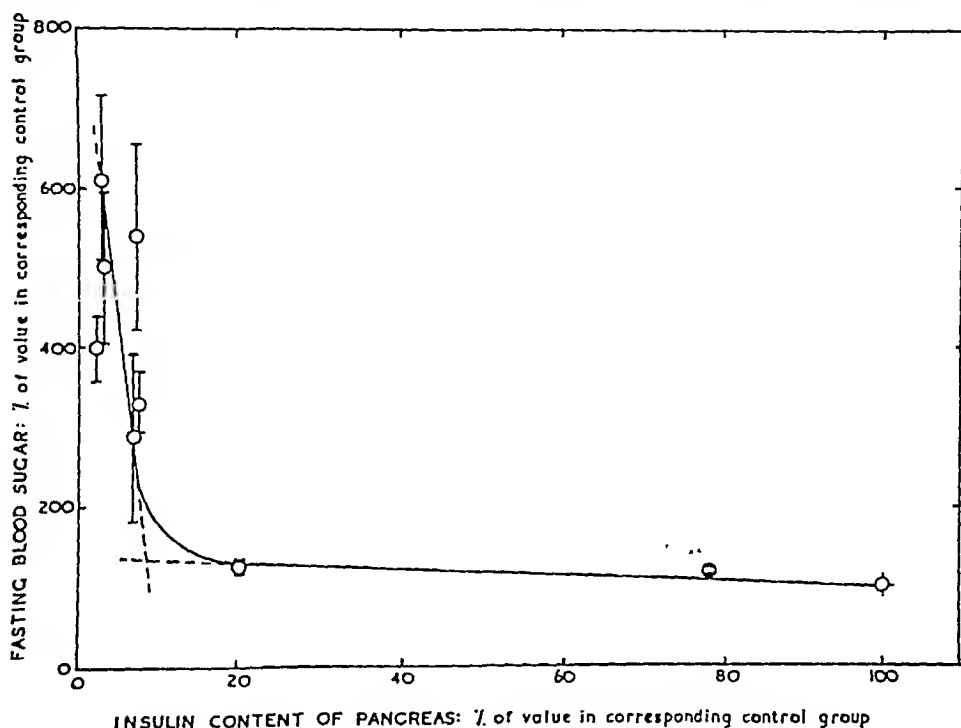


Fig. 4. RELATION BETWEEN INSULIN CONTENT of pancreas and blood sugar in fasted normal and alloxan-treated rats (*Experiment C*).

cells. Beta cells were clearly undergoing structural collapse and disintegration. Breakdown and removal of this central tissue produced what appeared to be a diminution in the average size of the islets. Some surviving beta cells were observed in pancreatic sections from representative animals in groups killed at 30 days which had shown transient glycosuria or were aglycosuric following alloxan injection (fig. 5B). The cytoplasm of these cells was reduced in volume and showed reduced granulation when compared with the beta cells of the normal controls. No histological difference was detected in the beta cells of either the aglycosuric or transiently glycosuric group, nor were mitotic figures seen.

In contrast to this the islet tissue of rats from *Group IVb* killed 72 days after alloxan was composed almost entirely of alpha cells. There is no evidence that the absolute number of these per islet had increased above that found in the normal controls.

GENERAL DISCUSSION

The results of *Experiment A* indicate that a period of partial or complete regression of urinary glucose excretion will occur in rats with recently-induced alloxan diabetes when maintained on a certain constant diet. In those animals in which the glycosuria was transient, the rate of glucose excretion can be represented moderately well as an exponential function of the time, the magnitude of the urinary glucose excretion rate falling to half-value in approximately six days in *rats 1, 2, 14, and 15*, and in a little more than two days in *rat 16*.

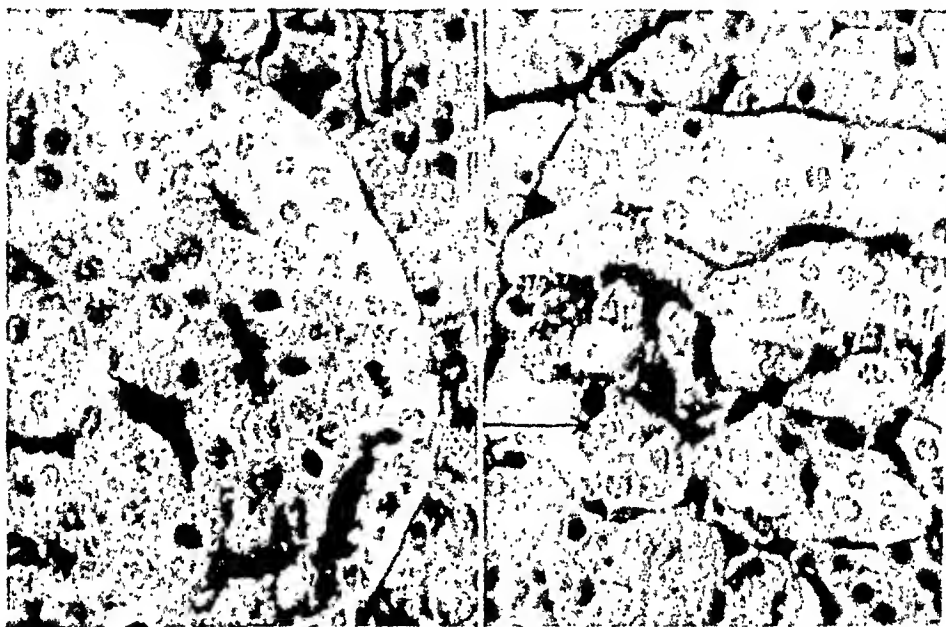


Fig. 5*A* (left). PARAFFIN SECTION (3μ) of pancreas of a normal rat, stained by McGregor's method (azo carmine, anilin blue and orange-G) following fixation with Bouin solution; $\times 700$. By this method the granules of the beta cells stain blue, and those of the alpha cells red. A portion of an islet of average size is shown. Note the relative numbers of the two types (beta cell granules appear dark, alpha cell granules pale) and the peripheral position of the alpha cells. *B*. (right). A preparation similar to that shown in *A*, but from the pancreas of a rat killed 30 days after subcutaneous injection of 200 mg/kg. of alloxan. This animal showed only a transient glycosuria. The insulin content of pancreases from similar animals was 19.8% of that in the corresponding control group. The arrow indicates a few persisting beta cells (dark).

The speculation of Burn, Lewis and Kelsey (7), that periods on a high fat diet might have been responsible for the regression of glycosuria observed in their experiments, is of great interest. While the diet used in the present experiments is limited in amount, it is definitely low in fat and high in carbohydrate content, and was fed continuously. The significance of the patterns of recovery from alloxan diabetes by rats, reported by several groups of experimenters, is obscured by lack of information on quantity of food ingested.

Janes and Prosser (8) found that alloxan-diabetic rats with long-standing glycosuria failed to become sugar-free when placed on high fat diets, although such a restriction was effective when the alloxan diabetes was of recent origin (7). In a series of observations on alloxan-diabetic adult rats which had been provided with Purina Fox Chow *ad libitum* for approximately two months we found no phase of

reduction in the total daily excretion of urinary glucose, such as is reported in this paper, when the ration was subsequently restricted to 13.0 gm/animal/day. The potential improvement in carbohydrate metabolism therefore appears to be limited to the first few weeks following alloxan administration.

The results of *Experiment B* show that the damage produced in the rat by alloxan is so rapid and extensive that the insulin remaining in the pancreas after 48 hours amounts to less than 10 per cent of the original depot. There is now reason to believe that the insulin content of pancreas at 48 hours in the alloxan-treated rat represents a fairly close approach to the minimum level through which it has been found to pass in this experiment and in *C*. Lazarow and Palay (9), and Cantor, Tuba and Capsey (10) have found that the 48-hour blood sugar level in the rat given single intravenous or subcutaneous doses of alloxan generally corresponds well with values measured during immediately succeeding days. Therefore, if the fasting blood sugar level in the rat can be expressed as a function of the insulin content of pancreas, as is indicated in figure 4, the insulin content at 48 hours does not differ significantly from the minimum level through which it passes after alloxan administration.

The relation between insulin content of pancreas and fasting blood sugar level in *Experiment C* is of interest in relation to a study made by Foglia (11) who observed that rats with 95 per cent of their pancreas removed developed diabetes only after several months. If it be assumed for the moment that insulin is uniformly distributed throughout the rat pancreas, Foglia's figure for the insulin content of pancreas at which diabetes becomes manifest appears to be somewhat lower than that indicated by our data, although both figures are of the same order of magnitude. Factors which might be responsible for the apparent difference would, on the one hand, include difficulties in measuring precisely the fraction of *islet* tissue originally present which was removed by partial pancreatectomy. On the other hand the presence of the exocrine pancreatic tissue has been shown to increase the insulin requirement of the alloxan-diabetic dog or that of the same animals with pancreatic ducts tied off above that observed in these animals after pancreatectomy (12-14). On this basis a difference in magnitude in the critical value of the insulin content of pancreas at which control of the fasting blood sugar is lost is to be expected. The results of Foglia's experiments and of ours indicate the existence of a large reserve of insulin-producing tissue in the normal rat.

The increase in insulin content of pancreas from the 48-hour level of 2.7 per cent of that in the corresponding normal control group fed the same ration to 19.8 per cent of the control value in the rats whose urinary excretion returned to normal in *Experiment C* is by itself suggestive of a restoration of capacity to produce insulin. The increase is accompanied by a significant fall in fasting blood sugar from 352 ± 35 mg. per cent to 108 ± 7 mg. per cent, by a return toward the control rate of increase in body weight, and by a highly significant fall in the excretion of urinary glucose. In *Group IVb* there is an indication of a moderate rise in insulin content of pancreas from 2.7 per cent to 7.9 per cent in the course of 72 days, and this is accompanied by a correspondingly moderate fall in average fasting blood sugar from 352 ± 35 mg per cent to 263 ± 37 mg. per cent, and by a slight decrease in excretion of glucose in the urine.

It has been demonstrated in *Experiment B* that administered insulin causes a reversible reduction in the urine sugar excretion by alloxan-diabetic rats. This observation supports the earlier reports of Shaw-Dunn and McLetchie (15) and of Cantor *et al.* (10) on the action of crystalline insulin on the blood sugar level of alloxan-diabetic rats. Hence the above observation of a spontaneous regression of urinary glucose excretion occurring coincidentally with an increase in insulin content of pancreas and a return toward normal fasting blood sugar levels suggests strongly that an increased rate of formation and release of insulin by the pancreas is the factor producing the regression. Such an interpretation is in full agreement with the basic principles upon which measurement of turnover rates are based, since the magnitude of the depot of material which is being turned over is one of two factors, the product of which equals the turnover rate (16). A more direct study of the turnover rate of insulin in pancreas is, of course, not as yet possible.

The histological observations of *Experiments A* and *C* are, broadly speaking, in agreement with those reported by Janes (17). The fact that the animals studied in his experiments were allowed to feed *ad libitum* while ours were not, adds an interesting variation to the study of the regression of alloxan diabetes in rats. The histological data support the view that the beta cells represent the component of the islets of Langerhans whose function is to produce insulin. These cells were severely necrosed by the alloxan at 48 hours, were found to be present in those alloxan-treated groups which retained or regained the condition of aglycosuria within 30 days, and appeared to be effectively absent from the group with sustained glycosuria when killed at 72 days. Furthermore, the degree to which recognizable beta cells were observed to be present in the islets of Langerhans corresponded on a qualitative basis with the insulin content of pancreas in the different groups.

A description of the mechanism by which restoration of the insulin content of pancreas and of the ability to utilize ingested carbohydrate occur in the alloxan-diabetic rat must await more definite information concerning the life cycle of the beta cells of the islets of Langerhans.

SUMMARY

Within 48 hours after subcutaneous injection of alloxan in diabetogenic doses to fasted adult rats, the insulin content of pancreas fell to only 2 to 10 per cent of the value in the normal controls.

The following types of response were observed to occur in adult rats receiving subcutaneous injections of 200 mg/kg. of alloxan, and subsequently maintained on a fixed ration: *a*) pronounced and sustained glycosuria; *b*) less pronounced and transient glycosuria, disappearing within 4 to 5 weeks following alloxan administration; and *c*) aglycosuria. Subnormal insulin content of pancreas values were found to exist in each of these types of animal several weeks after alloxan injection, the level being lowest for type *a* and highest for type *c*. In all three groups the insulin content of pancreas was found to be higher at time of sacrifice than that observed at 48 hours after alloxan. Recognizable beta cells with shrunken cytoplasm were observed in the islets of Langerhans of types *b* and *c* sacrificed 30 days after alloxan. None was identified in the islets of rats of type *a* sacrificed at 72 days after alloxan.

All of the data related to the incidence and regression of symptoms of alloxan diabetes in the rat as observed in the preceding experiments would be rationally accounted for if the changes in insulin content of pancreas could be taken as a measure of insulin turnover rate.

We are grateful to Professor C. H. Best for his guidance and encouragement, and to Miss Audrey Crooks for technical assistance, throughout the course of the experiments reported here.

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A POSSIBLE ROLE OF THE SKIN IN THE EFFECT OF ADRENALIN ON BODY TEMPERATURE AND RESPIRATORY METABOLISM

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ADMINISTRATION of adrenalin in physiological dosage to the intact animal invariably leads to increased respiratory metabolism and body temperature, which appear without discoverable dissent to be accepted as cause and effect, respectively. On the other hand, attempts to demonstrate a calorogenic action on isolated tissues have led to results so contradictory as to be completely inconclusive. In the face of this, the idea suggested itself that the uniformity of result in the intact animal might be due to a simple reversal of sequence: increased body temperature due to cutaneous vasoconstriction might be responsible, at least in part, for the increase in metabolic rate. As will be shown in what follows this appears to be true.

PROCEDURE

The experiments were made with fasting cats, anesthetized with dial-urethane (Ciba). Oxygen consumption was measured in a closed-circuit system from which carbon dioxide was removed by soda lime. Body temperature was recorded with a rectal thermometer. Freshly prepared adrenalin hydrochloride (Parke Davis) in saline was injected intravenously for 5 minutes at the rate of 0.004 mg/min/kg. body weight. Oxygen consumption was recorded continuously before, during, and for 25 minutes following the injection. Rectal temperatures were read each minute. Following a normal run, the skin was removed except from the head, feet and tail; all denuded parts were covered with vaseline and cotton. Rectal temperature, which fell during the operation, was brought back to the previous normal with artificial heat; when it and oxygen consumption were stabilized adrenalin was again injected and oxygen consumption and rectal temperature recorded as before.

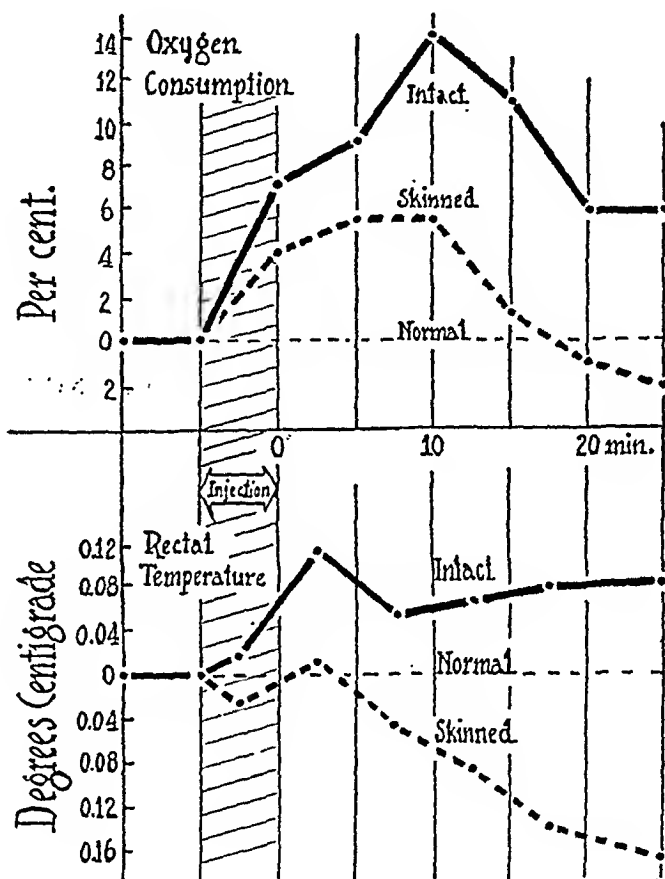
RESULTS

The cats fared well throughout the experimental period. Skinning caused little loss of blood, and had no apparent effect on pulmonary ventilation, blood pressure, pulse rate or the pressor response to adrenalin. In addition, it seemed to have no effect on normal oxygen consumption, the average for which, in the period preceding injection, was 12.6 in the normal and 12.9 ml/min. in the skinned animals. This small difference probably was related to the slightly unequal rectal temperatures

which as a consequence of failure exactly to control body temperature with artificial heat were 38.41 and 38.49°C . respectively.

Figure 1 shows the average results obtained on 14 animals. Oxygen consumption of the intact animal was increased 7 per cent during the 5-minute injection period and 14 per cent during the interval 5 to 15 minutes after injection; at the end of the experiment, 25 minutes after injection, it was still 6 per cent above the normal. These results gain credence by being almost exact replicas of a previous observation of the effect of the same dosage of adrenalin (1).

Fig. 1. THE AVERAGES of 14 determinations of the effect of adrenalin injected intravenously (femoral vein) at the rate of 0.004 mg/kg/min . on the oxygen consumption and rectal temperature of dial-urethane anesthetized cats before (solid line) and after removal of most of the skin (dotted line).



In the skinned animal oxygen consumption rose only slightly, 4 per cent during the 5-minute injection period. The maximum increase, which was only 5 per cent, occurred in the 10-minute interval following injection and was followed by a sharp fall to 2 per cent below normal 25 minutes after injection.

As to body temperature, the intact animal showed a rise of 0.03°C . during the 5-minute injection period; this continued to a maximum of 0.11°C . during the first 5 minutes after injection and was still 0.08°C . above normal 25 minutes after injection.

In contrast, the skinned animal showed an actual fall of temperature, amounting to 0.03°C ., during the injection period itself. A maximum rise to only 0.01°C . above normal occurred during the 5 minutes following the injection. This was succeeded by a steady fall, which persisted for the remainder of the experimental period, leading to a temperature 0.17°C . below the starting normal 25 minutes after injection.

DISCUSSION

These results appear to substantiate, at least in part, the hypothesis that cutaneous vasoconstriction in the intact animal by reducing loss of heat through the skin effects a rise of body (rectal) temperature. This, in turn, would be expected to increase the metabolic rate. In the skinned preparation there were no cutaneous vessels to constrict and so to conserve heat. Therefore if, as seems probable, the vessels in the splanchnic viscera were constricted as normally, the blood supply of the exposed striated muscles would be increased with the possibility of augmented heat loss. This could account for the general reduction, rather than increase, of rectal temperature and oxygen consumption in the skinned animals during and after injection. The source of the small increases which still occurred after skinning are being further studied.

SUMMARY

A skinned animal preparation is described which appears to be new and which may be useful in subsequent investigations. In this work the calorogenic response to adrenalin shown by intact cats is found to be reduced after the removal of the skin.

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EXPERIMENTAL PRODUCTION OF ELECTRICAL MAJOR CONVULSIVE PATTERNS

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EPILEPSY was described carefully in the writings of Hippocrates (1) and studied in the days of the Renaissance by Paracelsus, who suggested chemical anticonvulsant remedies. Modern investigation however began with Hughlings Jackson's neurological analysis (2) and the biochemical approach is still more recent. Thus, the study of pH changes, the inhalation of CO_2 and the water and electrolytic variations have contributed to our knowledge of these disorders. In the field of neurochemical investigation, the notion that certain kinds of convulsions might be ascribed to abnormalities of acetylcholine metabolism has proved to be a challenging concept.

While the exact rôle of acetylcholine in the nervous system is the subject of much controversy and debate, most investigators are agreed that this choline ester is of crucial importance in the function of the brain and is concerned in some manner with the nerve impulse. To be effective, acetylcholine must be released and destroyed with great rapidity. The enzyme, cholinesterase, rapidly hydrolyzes acetylcholine and is in this way intimately related to the action of acetylcholine. If the cholinesterase activity in a tissue is decreased acetylcholine will accumulate. Physostigmine and eserine are drugs that reversibly inhibit cholinesterase. During the time the enzyme is inhibited by these drugs acetylcholine may accumulate and included among the effects of excessive acetylcholine are convulsions. Fiamberti (3) is using intravenous injections of acetylcholine as shock therapy for schizophrenia and other conditions.

During the past decade several studies have been made on the action of acetylcholine on the brain in respect to the problem of convulsions. Among others, Miller, Stavrakys and Woonton (4) sensitized the brain with eserine and showed that acetylcholine could induce spiking in the brain waves. Later, Chatfield and Dempsey (5) prepared the brain with prostigmine and evoked spikes in the electrocorticogram. Atropine not only prevented this spiking but after this abnormal manifestation was established it could be eliminated by that drug. Brenner and Merritt (6) who applied acetylcholine to the cerebral cortex of the cat pointed out the similarity of the electrical changes resulting to those of grand mal epilepsy. Forster (7) continued along the same lines and was able to associate the electrical patterns with tonic and clonic convulsions. Bornstein (8) studied experimental cerebral trauma and showed that the resulting epileptiform discharges were accompanied by the presence of acetylcholine in the cerebrospinal fluid. The administration of atropine stopped these convulsant effects. In a clinical investigation Cone, Tower, and McEachern (9) showed that the cerebrospinal fluid of patients either in status epilepticus or with more than one seizure per day contained significant amounts of acetylcholine.

During the last few years an irreversible anticholinesterase has been discovered, di-isopropyl fluorophosphate (DFP). This drug affords an ideal means of studying the effects of excess acetylcholine in the brain for unlike eserine and prostigmine which temporarily inactivate cholinesterase; DFP destroys that enzyme (10, 11).

Previous contributions include that of Grob *et al.* (12) who administered DFP to man and observed encephalographic changes consisting of an increase in the potential, frequency and irregularity of rhythm, and the intermittent appearance of abnormal waves similar to those seen in patients with grand mal epilepsy. Wescoe *et al.* (13) injected DFP intravenously and produced convulsions with a constant electroencephalographic effect characterized by an increase in frequency and a decrease in voltage. However, in neither case was status or repetitive high amplitude rapid frequency waves of an enduring grand mal seizure seen. Such electroencephalographic patterns were observed in the following experiments.

METHOD AND RESULTS

Rabbits were anesthetized with the ultrashort-acting barbiturate, pentothal, given intravenously, but were maintained under anesthesia only long enough to expose the vessels of the femoral triangles and the two common carotid arteries, as well as to cannulate the trachea and resect the scalp. Electrocardiograms were obtained from each cerebral hemisphere by means of monopolar needle electrodes inserted through the calvarium (14). For each hemisphere the corresponding external auditory meatus was used for the insertion of an indifferent electrode. A four-channel Grass electroencephalograph was employed to record the electrical changes. Prior to the administrations of DFP a small dose of atropine sulfate, 0.02 mg/kg., was injected into the femoral vein to prevent bradycardia and maintain the normal hemodynamics of the animal. Electrocardiograms were recorded throughout the experiments in order to follow the heart function. Furthermore, to be sure physiological conditions prevailed, blood pressure readings were obtained intermittently from the femoral artery. After the slow pentothal waves had disappeared, DFP in saline solution was injected into the right common carotid artery of curarized rabbits under artificial respiration. The first step in the elucidation of the mechanism of the convulsives is to study the action of DFP on cholinesterase. Cholinesterase activity was therefore determined by a potentiometric technic developed by Michel (15), on slices from the right and left hemispheres, the cerebellum and the area termed brain stem, starting with the thalamus and extending caudally through the corpora quadrigemina.

In figure 1 are presented the characteristic stages in the development of the grand mal pattern produced by the intracarotid injection of DFP into a rabbit. The progressive changes include first an increase of frequency and a decrease in amplitude followed by a gradually enlarged amplitude. Despite the anatomic presence of the circle of Willis the injection of DFP into the right common carotid artery goes predominantly to the right side of the brain (16), and for that reason the left side lags behind the right. Nevertheless, with adequate doses the left side finally also developed the grand mal pattern. As indicated in the legend the larger doses of DFP evoked the more marked effects. However, a certain overlapping of dosage was observed, for example 1.0 mg/kg. produced *stage C* in one animal but *stage D* in another and on several occasions a single dose of 0.5 mg/kg. brought forth the entire gamut of changes culminating in the grand mal pattern as seen in the tracings presented in figure 1. However, in any given animal the development of grand mal

potentials with adequate amounts of DFP was consistent. This pattern, once established, was maintained for $\frac{1}{2}$ hour to $1\frac{1}{2}$ hours.

Certain variations appearing consistently bring to mind the electroencephalographic records seen in epilepsy states other than typical grand mal. Thus with the injection of 0.5 mg/kg. of DFP a pattern similar to that described by Gibbs and

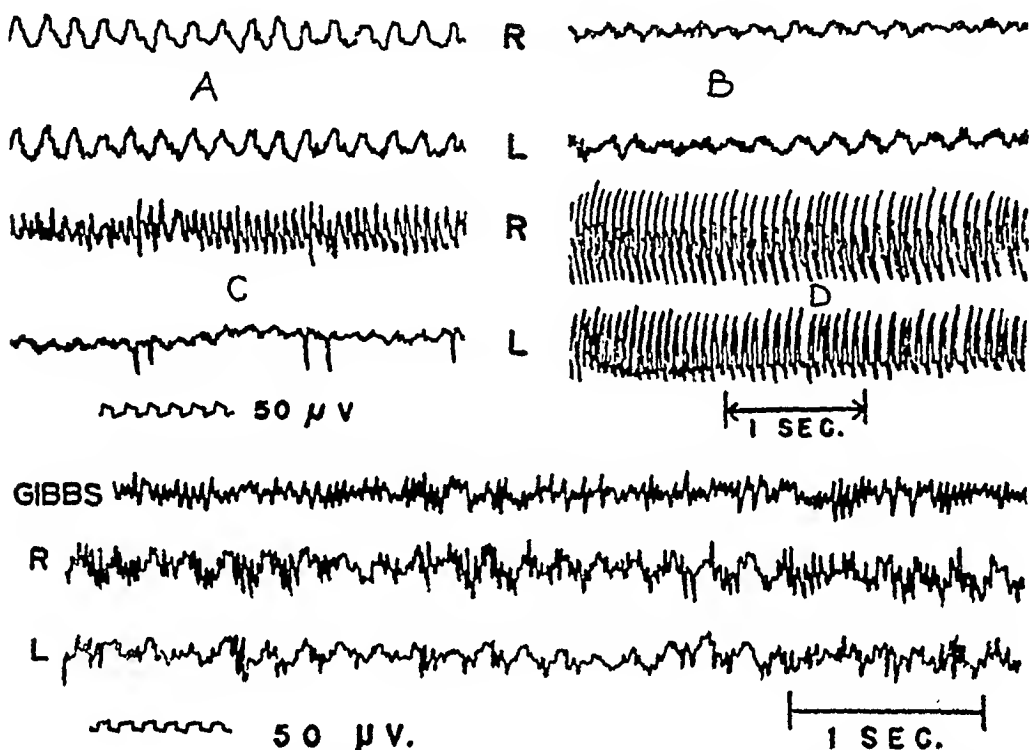


Fig. 1 (*upper*). DFP AND ELECTROCORTICOGRAM. Control tracings for right and left hemispheres, frequency 5/sec., are presented in A. The first change, B, includes the appearance of secondary waves superimposed on slightly increased fundamental frequency on the right to 6/sec., and a decrease in amplitude more marked on right than left. Such changes occurred with dosages of about 0.2–0.5 mg/kg. The second or intermediate change, C, consists of a grand mal type of wave, frequency 15/sec. with increased amplitude in the right hemisphere while the left hemisphere advances to the characteristics of the right hemisphere during the preceding change with, however, an occasional spike. Electrocoorticograms of this type appeared with doses of 0.5–1.0 mg/kg. The third change, D, resembles the pattern of status epilepticus and was observed in both hemispheres. The frequency was 19/sec. and the amplitudes the largest observed. The dosage with which grand mal pattern developed was usually 1.0–2.0 mg/kg.

Fig. 2 (*lower*). PATTERN OF TONIC SEIZURE COMPARED WITH DFP EFFECTS. Tracing marked Gibbs is one of a tonic seizure reproduced from the Atlas of Gibbs and Gibbs (17). Those below are the right and left electrocorticograms obtained in a rabbit injected with DFP. The development of the electrocorticogram of the left side lags behind that of the right.

Gibbs (17) for tonic seizures was observed (fig. 2). These authors note that such changes may precede grand mal. We have made the same observations for this type of wave occurs after the earlier reactions take place and before the grand mal tracings are established. Like *stage C* in figure 1 and the type of waves shown in figure 3, those presented in figure 2 therefore belong to the group of intermediate changes. More frequently and almost always before the repetitive grand mal seizure commenced, rectangular high amplitude waves, which are sometimes serrated and typical of psychomotor seizures, appeared in the electrocorticograms (fig. 3).

While grand mal seizure patterns were disappearing, either spontaneously or because of anti-epileptic treatments, clumps of high frequency spikes could be seen interspersed between less abnormal tracings (fig. 4). Similar configurations have been observed in patients with myoclonic seizures as concomitants of epilepsy.

Biochemical investigations on the brain of the rabbit studied electrically reveal (table 1) that a marked depression of cholinesterase activity occurred before any definite changes were observed in the electrocorticogram. With this drug an

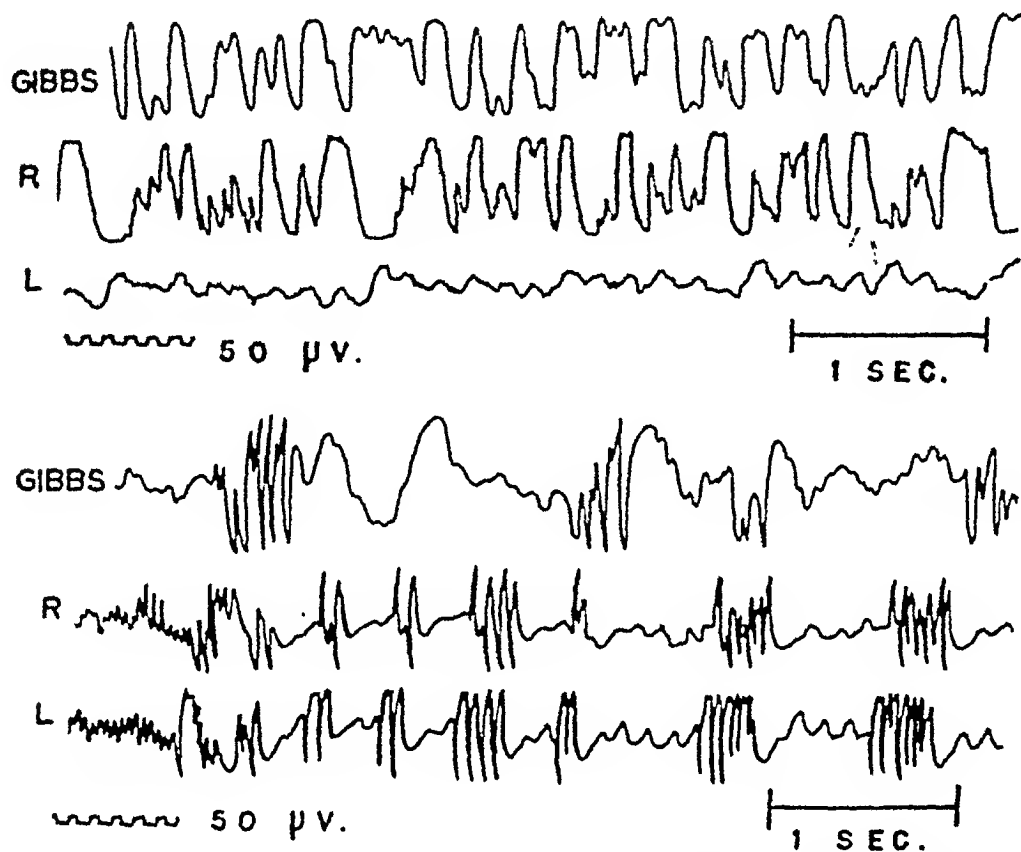


Fig. 3 (*upper*). PATTERN OF PSYCHOMOTOR SEIZURE COMPARED WITH DFP EFFECTS. First tracing, obtained on a patient with psychomotor seizures, is reproduced from the Atlas of Gibbs and Gibbs (17). The right electrocorticogram observed in a rabbit is similar to the tracing from the Atlas. The left side again lags behind the right.

Fig. 4 (*lower*). MYOCLONIC PATTERN COMPARED WITH DFP EFFECTS. Top pattern is reproduced from the Atlas of Gibbs and Gibbs (17) and was obtained from epileptic patients who have exhibited myoclonic seizures. The lower two tracings are reproduced from a rabbit injected with DFP.

extreme depression of cholinesterase activity is an accompaniment of the grand mal pattern. In none of the observations, however, was the cholinesterase activity brought to a complete standstill, some activity always survived even with the largest doses of DFP used.

With a decrease of cholinesterase activity acetylcholine presumably accumulates and in that case atropine should be a potent antidote. In order to test the influence of atropine, in three instances that drug was given after the grand mal waves had been established (fig. 5). Surprisingly small doses of atropine for the rabbit restored the normal pattern. Even 1 mg/kg. of atropine exerted an enormous influence, 2

to 4 mg/kg. were the most required to eliminate the electrical alterations. In four other observations atropine was injected intravenously in dosages varying from 6 to 25 mg/kg. prior to the administration of DFP. Though the grand mal waves were prevented, DFP evoked some moderate responses such as increases in frequency and

TABLE 1. CHOLINESTERASE ACTIVITY AND ELECTROCORTICOGRAM

ELECTROCORTICOGRAM	CHOLINESTERASE ACTIVITY % NORMAL	
	Right cerebral hemisphere	Left cerebral hemisphere
Early change.....	8.7	55.0
Intermediate.....	0.8	8.0
Grand mal.....	0.6	1.6

Following the right intracarotid injection of a small dose of DFP, 0.1 mg/kg., the earliest electrical changes characterized by slight increase of rate and diminution of amplitude appearing only in the right hemisphere was observed. The right cortex was 8.7% of normal and the left 55.0%. With larger doses of DFP, usually 0.5 mg/kg. to 1.0 mg/kg., intermediate changes in the electrocorticoqram were seen, such as bursts of low amplitude fast waves with occasional high square waves on the right and low amplitude waves on the left. In this instance the right cortex had a cholinesterase activity of 0.8% of normal and the left 8.0%. Lastly, the electrical pattern of grand mal was found to be accompanied by 0.6% of the normal cholinesterase activity on the right and 1.6% on the left and the dosage varied from 0.5 to 2.0 mg/kg.

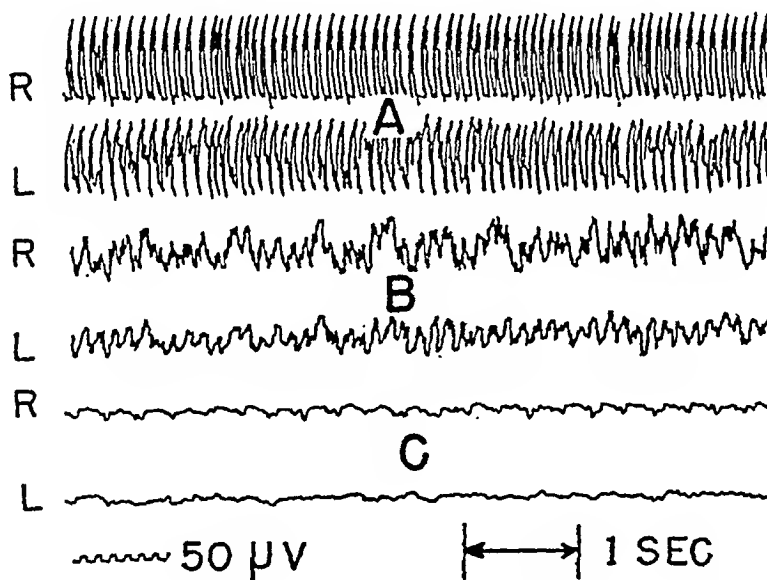


Fig. 5. DFP AND ATROPINE CURE. The first tracing, A, is that of a grand mal seizure pattern evoked by the intracarotid injection of DFP. The second marked B shows the influence of the intravenous injection of atropine, 1 mg/kg., on the seizure pattern. The third, C, exhibits the effect of an additional dose of atropine, 1 mg/kg.

decreases in amplitude. These responses were however eliminated by additional atropine.

DISCUSSION

The reproduction of the electrical patterns similar to those observed (figs. 1-5) in patients with epilepsy has been accomplished in rabbits receiving intracarotid

injections of DFP. In order to evoke the convulsive patterns drastic reductions of cholinesterase are required (table 1). These experiments therefore suggest that excessive acetylcholine is concerned with the experimental production of the grand mal type of electrocorticogram. It is true that several groups of investigators (18-22), have reported actions of DFP other than the destruction of cholinesterase. The ability of this drug to destroy cholinesterase however is generally recognized and in the absence of the hydrolytic enzyme acetylcholine may accumulate.

In favor of this interpretation of the rôle of acetylcholine are the effects of atropine. If these abnormal tracings were brought on by an excess of acetylcholine a drug that blocks the action of acetylcholine should prevent or cure these seizures. This therapeutic action is seen in the present experiments.

Though the present studies do not present evidence on the concentration of acetylcholine in the various parts of the brain, an attempt is made to correlate the electrical potentials with decreases of cholinesterase activity. This comparison is facilitated because DFP produces a biochemical lesion in the brain which endures until regeneration of cholinesterase occurs. A comparison of table 1 and figure 1 reveals a rough correlation between the degree of abnormal electrical potentials and the depletion of cholinesterase activity, for with the progressive decrease of the latter the electrocorticograms exhibit successive stages in the production of the grand mal pattern. This relationship however is not necessarily a direct one and may involve other factors.

Experimentally produced convulsions, in many instances, do not respond to anti-epileptic drugs in the same manner as does essential epilepsy (23-25). Among the various experimental methods for testing and screening anti-epileptic drugs, i. e. electroshock and metrazol, none gives adequate assurance as to effectiveness of the drug in question. It is, therefore, of interest to report that our preliminary results indicate a similarity between the influence of the anti-epileptic drugs on DFP and clinical experiences insofar as phenobarbital, dilantin and tridione are concerned.

What is the relation of these electrical patterns to those of essential epilepsy? There are certain bits of evidence in the literature indicating that acetylcholine may enter somehow into the mechanism of essential epilepsy. Gibbs, Lennox and Gibbs (26) have observed increases of carbon dioxide in the arterial blood of patients reaching maximum values just before the precipitation of the convulsions. An accumulation of acetylcholine might be expected at that time in view of Finerty and Gesell's (27) work emphasizing the anticholinesterase action of carbon dioxide. Supporting this possibility is the conclusion of Wortis and Goldfarb (28) who studied patients receiving the hypoglycemic treatment for schizophrenia and observed that the arterial carbon dioxide rose sharply preceding the onset of convulsions. In this regard the observations of Schütz (29) that patients habituated to barbiturates reveal a decrease in the nonspecific cholinesterase activity of the serum may be cited. On the removal of the habituating drug convulsions were frequently observed. A low cholinesterase activity accompanied by a corresponding excess of acetylcholine with the elimination of the depressing neural effect of the barbiturate may be among the causes for the convulsions. Other suggestive evidence is that Pope *et al.* (30) who excised electro-

genic areas from patients with convulsions found excessive cholinesterase activities. They believe that the supernormal activity is indicative of an abnormally large acetylcholine turnover.

We need scarcely point out that similarity does not establish identity and that these experiments are not necessarily concerned with the mechanism of epilepsy. Atropine moreover is not effective in the management of epilepsy, and yet a recent report (12) indicates that it may exert a beneficial effect on some of the abnormal waves appearing between convulsive episodes. Unlike many other convulsants, in the case of DFP, the active agent is a physiological one namely acetylcholine. Thus there is a likelihood that this mechanism might play a rôle in clinical seizures.

SUMMARY

Intracarotid injection of DFP into curarized rabbits produced high amplitude rapid frequency waves on the electrocorticogram similar to those seen in grand mal seizures of status epilepticus. Various patterns were demonstrated that resemble other epileptic tracings. These electrical changes were associated with extreme decrease of cholinesterase activity but in no instance was all such activity eliminated. Because of the destruction of cholinesterase it is probable that the convulsant patterns are produced by the excessive accumulation of a normal metabolic product of the brain, namely, acetylcholine, an interpretation supported by the prevention of the seizure patterns by the injection of atropine.

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DFP: SITE OF INJECTION AND VARIATION IN RESPONSE

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THE drug, di-isopropyl fluorophosphate, called for brevity DFP, irreversibly inactivates cholinesterase after a brief latent period (1, 2). With the depression of cholinesterase activity, acetylcholine may accumulate in the body and the signs of DFP activity are in large measure the results of excessive acetylcholine. In addition to combining with cholinesterase, DFP may undergo another fate, namely, to be destroyed by an enzyme which Mazur and Bodansky (1) named phosphofluorase, an enzyme present throughout the body and perhaps in greatest concentration in liver and kidney.

The symptoms and the lethality of DFP may be expected to vary in accordance with the site of injection. If the DFP goes chiefly to an organ sensitive to acetylcholine the results of the injection will be magnified. While if most of the drug enters an organ containing a high concentration of phosphofluorase it will be detoxicated rapidly and its action will be minimized.

When a drug is injected intravenously it will necessarily be carried to the heart from whence it will be spread to the rest of the body and affect all the organs. On the other hand, the action of a drug on any particular organ can be segregated to some extent by the injection of that drug into an afferent vessel of that organ. For most parts of the body their arterial supply would be the logical avenue of entrance. In the liver, however, the portal vein may be substituted for the hepatic artery as an easier site of injection. Finally, as a quantitative means of accurately estimating one of the actions of a drug, namely, its lethality, the LD₅₀ resulting from the various sites of injection can be determined.

METHODS AND RESULTS

In the present study made on rabbits the animals were anesthetized with the ultrashort-acting barbiturate pentothal given intravenously. The animals were maintained under anesthesia only long enough to expose the vessels to be injected. All animals were examined for the changes in behavior produced by DFP. When a lethal exitus occurred it too was noted and the time recorded. The sites of injection were: *a*) both common carotid arteries, *b*) one common carotid artery, *c*) femoral vein, *d*) femoral artery and *e*) portal vein. In order to segregate electrical activity of each cerebral hemisphere two monopolar electrodes (7) were inserted through the cranium, one over each cerebral hemisphere. Each monopolar electrode was connected with an indifferent electrode placed in the external auditory meatus of the same side of the head. The animals were curarized, placed under artificial respiration and the electrical potentials of the cerebral hemispheres recorded. Subsequently, the cholinesterase activities of brain slices were measured by the method of Michel (3).

Table 1 presents the results on the lethality of DFP and LD₅₀ is recorded for

each site of injection. With the exception of the LD_{50} values for one carotid artery and the femoral vein, each LD_{50} is significantly different from every other one according to the t test.

The effects on the behavior of the animals depended upon the site of injection. When the femoral vein was used general effects were strongly developed including gastro-intestinal hypermotility and defecation with some stimulation to respiration. Injection of a femoral artery invariably resulted in lively fasciculations of the leg muscles fed by the artery. The general effects however were less noticeable than with the femoral vein. In the case of the portal vein all signs of DFP poisoning were profoundly minimized. The use of the common carotid artery for the administration of DFP invariably produced marked respiratory stimulation and in the end respiratory depression. Salivary secretion was another prominent result.

In some of the animals injected in only one common carotid artery a characteristic unilateral or adverse syndrome developed. For example, if the left common carotid artery was used for the administration of DFP the pupil of the left side constricted, the head turned to the right and compulsive circling movements to the right were made. The behavior of the animals suggests a labyrinthine component of the syndrome.

TABLE 1. INJECTION SITE AND DFP SENSITIVITY

SITE	LD_{50} MG/KG.	RATIO	SITE	LD_{50} MG/KG.	RATIO
Both carotids.....	0.109 ± 0.030	1	Femoral art.....	0.858 ± 0.082	8
One carotid.....	0.456 ± 0.061	4	Portal vein.....	2.30 ± 0.15	22
Femoral vein.....	0.478 ± 0.063	4			

DISCUSSION

It will be seen that the brain is the organ most sensitive, for the LD_{50} dose is only 0.109 mg/kg. when both carotid arteries are injected simultaneously. In that case respiratory signs are especially pronounced for the animals so treated display marked hyperpnea followed by diminished respiration cyanosis and finally apnea if the outcome is lethal. Usually the heart continues beating after respiration has ceased. In our experiments the effects on respiration might have been exerted at least in part indirectly through the orbital surfaces of the frontal lobes, for cholinesterase activity was reduced most profoundly in the cerebral hemispheres. On stimulation of these surfaces Sachs and Brendler (4) observed respiratory arrest in the expiratory position and inhibition of respiration in any phase of its development. The same respiratory changes may also be evoked either by the vagus or by the medullary centers. Frey and Gesell (5), using the vertebral artery for injection in order to reach the respiratory centers directly describe the mechanism whereby increasing doses of DFP produced first greater ventilation, then a decrease and finally apnea. Just as in the case of vagus stimulation, the expiratory centers undergo a progressive increase of function under the influence of DFP. The inspiratory centers are reciprocally inhibited more and more profoundly so that inspiration at first is shortened and finally prevented entirely as respiration ceases in the expiratory

phase. With bilateral intracarotid injection the systemic effects are relatively small. It seems that the DFP is filtered out, to a greater or lesser extent, by the brain and other parts of the head. In addition the longer circuit in the blood permits the combination of a greater amount of DFP with the cholinesterase of the red blood corpuscles.

When only one carotid artery was used, a dose four times as large as the bilateral route was required. The injection of one carotid artery goes preponderantly to the same side of the brain leaving the opposite side less affected. The electroencephalographic evidence for the lack of good mixture of blood in the brain will be considered later. Preliminary observations reveal that the changes in posture and forced circling movements named the adverse syndrome may be elicited whether the internal or external carotid artery is injected with DFP, and can be cured with atropine in doses of 0.7 to 0.8 mg/kg.

The LD_{50} for the femoral vein is about the same as for a single carotid artery. Though the brain may receive less DFP than when only one common carotid artery is injected yet the dose is not diverted to one side of the head but is distributed more equally to all parts of the body. The general systemic signs including peristalsis and defecation are therefore more prominent while those arising in the head, i.e. pupillary constriction, salivary secretion and respiratory stimulation, are less so. The respiratory changes must be considered in part as the direct action of DFP on the lungs with contraction of bronchial musculature and secretion of bronchial glands. The use of the femoral artery as an avenue for the administration of DFP necessitates a larger dose than is required when the femoral vein is so employed because the drug must first pass through the leg making for the retention of that agent within the muscles. A sign of this retention is the highly localized increase of muscular activity particularly in the form of fasciculation in the injected leg. Thus, some of the DFP is diverted from the rest of the body.

Finally, the injection into the liver via the portal vein requires a dose all out of proportion to that of any other chosen sites probably because of the high concentration of phosphofluorase in that organ (1) which rapidly detoxicates the drug and correspondingly raises the LD_{50} .

In general these experiments show that the effect of a given dose of DFP varies widely with its site of entry into the body. If we take the dose for the two carotid arteries as unity then table 1 shows that the relation of the two common carotid arteries to one artery is as 1:4, the two common carotid arteries to the femoral vein is also 1:4, to the femoral artery, 1:8 and to the portal vein, 1:22.

Because the brain is the organ most sensitive to DFP it was decided to study further the brain changes with the aid of the electroencephalograph (6). Monopolar electrodes were therefore inserted through the cranium over each cerebral hemisphere (7). When doses of DFP less than the LD_{50} were administered into a common carotid artery of curarized artificially respired rabbits, rapid low amplitude waves appeared in the electroencephalogram. This electrical change was exhibited chiefly though not exclusively on the side of the injection. With larger doses, one to three times the LD_{50} , the grand mal type of wave was evoked and in this case finally both sides of the brain were equally involved. The influence of atropine on the results

of DFP are significant. Atropine was able to restore the normal pattern after major convulsive seizures appeared on the electrocorticogram. Moreover, when atropine was given before DFP it prevented the appearance of the grand mal pattern but could not entirely eliminate short periods of the fast, small amplitude waves (6).

Though there is no consensus of opinion on the possible mechanisms of action of DFP yet general agreement does exist on its ability to attack the enzyme cholinesterase. For that reason the cholinesterase activities of various parts of the brain were determined. Elsewhere (6) it is reported that with appropriate small doses of DFP the cholinesterase activity of the injected side was more impaired than the opposite one. But with the larger doses ranging from 0.5 mg/kg. to 2.0 mg/kg., productive of bilateral grand mal patterns, a profound depression of cholinesterase activity was observed in both cerebral hemispheres. Such a depression permits the accumulation of excessive acetylcholine which in turn may be an immediate cause of the convulsant electrical potentials.

Finally, the anatomic basis is at hand for the more profound effects observed on the side of the intracarotid injection of DFP. The differences between the results of bilateral and unilateral intracarotid injections whether measured in terms of lethality, behavior, brain waves or cholinesterase activities must be directly associated with the absence of the anterior communicating artery which appears for the first time in phylogeny among the primates. In the rabbit, the anterior cerebral arteries, branches of the internal carotid arteries, are not connected by the anterior communicating artery. Rogers (8) has shown that the circle of Willis in man does not facilitate the mingling of the blood streams of the vertebral and carotid arteries. The circle is neither a distributor nor an equalizer. His conclusion applies with even greater force to the rabbit for in that species the circle is incomplete.

SUMMARY

A study of lethality with different sites of injection has shown the brain to be the part of the body most sensitive to DFP, the cause of death being respiratory. Among the other organs, the liver managed the largest doses of DFP with least general effects. Injection into one common carotid artery produced an adverse syndrome consisting in part of forced circling movements to the side opposite the injection. When artificial respiration was instituted in order to avoid respiratory failure large doses of DFP precipitated grand mal type of brain waves. The subsequent injection of atropine restored the normal pattern. Atropine given before DFP prevented the development of the grand mal pattern.

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WATER, NITROGEN AND ELECTROLYTE CONTENT OF BRAIN FOLLOWING CEREBRAL CONCUSSION¹

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FOR some time the belief has been that cerebral swelling is an important factor in producing the symptoms following trauma to the brain. Therefore a study of the biochemical changes in the blood and cerebral tissues in animals in which concussion is produced experimentally should be of value.

The purpose of this investigation was to study the water, nitrogen and electrolyte concentrations in the serum and brain of dogs after pure cerebral concussion according to the technique developed by Walker, Kollros and Case (1). The discussion will be confined to the effect of concussion on water and electrolyte values in the brain of dogs grouped according to the length of time allowed to elapse between the impact of the blow and the removal of the brain for analyses. The study includes the analytical data from 32 dogs apportioned as follows: 12 normal dogs used as controls; 4 animals at 15 minutes after trauma; 4 animals at $1\frac{1}{2}$ to $3\frac{1}{4}$ hours after trauma; 6 animals at 5 hours; 3 animals at 24 hours; and 3 animals at 48-72 hours. These intervals of time that were allowed to elapse between the impact of blow and the removal of the brain were selected to determine whether there were detectable post traumatic changes and, if so, the time necessary for their development.

Many studies have been carried out to attempt to establish causes of symptoms following injuries to the head, and numerous reviews of the literature have been published on this subject. The most critical and extensive one was that by Lehman and Parker (2). Another early review was that by Pilcher (3), who presented data to show that the water content of various portions of the dogs' brain did not change after measured trauma of varying degrees. Since the total water content did not change he concluded that, following trauma, cerebral edema was not the important factor in producing the increased intracranial pressure. Later in 1943, Gurdjian, Webster and Arnkoff (4) reviewed conditions which cause the brain to swell. The recent work of White, *et al.* (5) established that, when cerebral concussion was produced in cats, there was an increase in the volume of the brain up to a maximum of 5.5 per cent. They stated that the increase in brain value was due to extravasation of fluid through the capillary walls rather than to the escape of red cells, or vascular congestion or distension of the ventricles with increased amounts of fluids.

There have been no known experimental studies on the water and electrolyte content of the cerebral tissues following trauma. It was considered possible therefore that chemical examination of the cerebral tissues following pure concussion might

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reveal changes not recognizable by morphological study of the tissues alone. The experiments reported here deal primarily with the chemical changes in cerebral tissues obtained from dogs with pure cerebral concussion, accompanied by minimal damage to blood vessels.

PROCEDURES

The dogs used as the experimental animals weighed about 8 kg. and were in good physical condition. For the concussion and the removal of tissue all animals were anesthetized with pentobarbital. In brief the procedure was as follows. The skull was exposed and a hole trephined in the right parietal area to accommodate a right-angled plumbing elbow. With the elbow in place a five-foot metal tube was screwed into it and the elbow and tube were filled with water to a height of about two inches. The impact of a falling weight, generally a 140-gm. steel weight, upon the surface of the water was transmitted to the dura and then to the brain. The severity of the concussion varied. Some dogs simply skipped one or two respirations. Others had respiratory arrest for a minute or more and their corneal reflexes disappeared for still longer periods. A few maintained a tonic state for several minutes, relaxing slowly and gradually as artificial respiration was instituted. When the desired time had elapsed after the concussion, the animals were anesthetized with pentobarbital (nembutal) and blood and brain were removed.

Blood was drawn under oil from the femoral artery and allowed to clot. The serum was used for the analyses of water, chloride, sodium, potassium, calcium, magnesium and total nitrogen. Immediately after withdrawal of the blood, the brain was removed. The brain was wiped quickly to remove adhered blood. The brain stem and cerebellum were separated by section through the peduncles at the level of the superior colliculus and the cerebral hemispheres divided in the sagittal plane. The hemispheres and the cerebellum were then placed in separate glass stoppered bottles, treated and analyzed as previously reported by Eichelberger and Richter (6). The brains of all animals were carefully examined in order to be certain that there were no intracranial hemorrhages or any other pathologic alterations detectable to the naked eye.

The following determinations were made on these tissues: water, chloride, sodium, potassium, calcium, magnesium and total nitrogen. The chemical methods used are described in the above reference (6).

RESULTS

For comparison, the mean data with standard deviations for normal serums, hemispheres and cerebellum are given in table 1A for 12 dogs. Since it is impractical to determine the water and electrolyte content of the same cerebral tissues before and after trauma, the available basis for comparison is the average findings in a group of normal animals. In using this procedure, the effects of individual variations between different brains are not eliminated, although this limitation is obviated by the small standard deviations (table 1) for all the constituents studied.

GROUP I. *Analyses of cerebral tissues removed 15 minutes after trauma.* In table 1B are recorded the quantitative chemical concentrations present in the serum, hemi-

sphere and cerebellum of 4 dogs, 15 minutes after concussion. The average values of the 4 dogs are presented together with the calculated difference between the experimental values and the control values under the heading of change. In general, when the average findings of this group of dogs are compared with the averages of the control group, the average change does not indicate any significant difference between 'control' and experimental tissues for a 15-minute period. The reason for the absence of any changes in this group of animals may be the result of the relatively short time between the blow and the removal of the brain. Therefore longer intervals were allowed to intervene after the impact.

GROUP II. *Analyses of cerebral tissues removed $1\frac{1}{2}$ hours to $3\frac{1}{4}$ hours after trauma.* In table 1C are given the chemical analyses for serums and cerebral tissues of the 4 dogs forming this group. Three of the animals were killed 3 hours and one, $1\frac{1}{4}$ hours after the blow. The only material change observed in the chemical constituents of the tissues of this group of animals was the low calcium content in the hemispheres of 3 of the 4 dogs. In dog 5 of this group, an extremely low calcium value of 1.04 mEq. of calcium/kg. of hemisphere was obtained.

GROUP III. There were 6 animals in this group, 2 were killed at the end of $4\frac{3}{4}$ hours; 2, at the end of 5 hours and 2, after $5\frac{1}{4}$ hours following the cerebral injury. The results are presented in table 1D. The only significant chemical change in this group of animals was the low calcium values in dogs 13 and 14.

GROUP IV. *Analyses of cerebral tissues removed 24 hours after trauma.* Three animals comprised this group and the results are given in table 1E. The concentrations of both sodium and chloride in the serum were low, especially the sodium values. The cerebral tissue values for chloride, sodium and calcium were also low. The water content of neither the serum nor cerebral tissues was altered. Low calcium values were found in all dogs of this group.

GROUP V. *Analyses of cerebral tissues removed 48 to 72 hours after trauma.* One dog was killed 48 hours after trauma and 2, 72 hours after the blow (table 1F). Chemically, the serum and cerebral tissues of these dogs were normal. Although changes occurred in the tissues of the Group IV dogs 24 hours after concussion, they were not detectable in the group examined at 48 to 72 hours following trauma.

DISCUSSION

The major difficulty in determining whether or not the cerebral tissues swelled following pure concussion is that it is impossible to calculate from the available data the relative volumes of the extracellular fluid phase and the intracellular fluid phase. If we consider that brain tissue, as all other tissues, comprises two phases, the extracellular fluid phase and the intracellular fluid phase, we should accept that the extracellular space of the central nervous system is distinct in character from that of the extracellular space of other tissues. This conclusion was reached by Wallace and Brodie (7) and Manery and Bale (8) on finding that the passage of dissolved substances from the blood into the extracellular space of the central nervous system is slow while into the extracellular spaces of all other tissues it is rapid.

Generally, if a tissue swells, one of the following conditions has occurred: a) The total water content of the tissue has increased with a resultant increase in one of the

TABLE I. CONCENTRATION OF CONSTITUENTS OF SERUM AND CEREBRAL TISSUES OF DOGS
FOLLOWING CEREBRAL CONCUSSION

DOG. NO.	TISSUE	H ₂ O	Cl	Na	K	Ca	Mg	TOTAL N	BLOWS TIME AP- PER BLOW
		gm/kg.	mEq/kg.	mEq/kg.	mEq/kg.	mEq/kg.	mEq/kg.	gm/kg.	hr.
<i>A. Normal Control Dogs</i>									
	Serum	973.6±6.4	108.8 ±4.4	141.4±2.8	4.66±0.24	4.04±0.32	1.99±0.16	9.84±0.42	
	Hemisphere	761.3±8.3	36.71±1.05	51.0±2.4	95.6 ±4.7	2.14±0.14	11.26±1.12	18.0 ±0.3	
	Cerebellum	745.0±7.0	35.19±0.89	50.8±1.7	92.7 ±4.0	2.14±0.14	10.80±0.60	19.1 ±0.5	
<i>B. Group I. Brain Removed 15 Minutes After Trauma</i>									
1	Hemisphere	770.0	37.34	53.7	101.5	2.38	11.62	19.0	1
	Cerebellum	766.0	36.00	49.5	98.8			19.6	
2	Serum	935.1	109.2	139.4	3.15	5.00			3
	Hemisphere	777.5	38.01	53.6	109.5	2.42	12.08	18.9	
	Cerebellum	757.2	39.34	57.5	105.2			19.6	
3	Serum	928.3	114.2	139.6	4.52	4.96		9.07	1
	Hemisphere	759.5	34.87	50.1	104.2	2.58	9.58	18.7	
	Cerebellum	741.4	34.93	49.7	95.4			19.6	
4	Serum	924.1	108.4	139.5	4.82			8.95	1
	Hemisphere	775.2	37.67	51.4	102.7	2.06	8.94	18.3	
	Cerebellum	745.0	35.87	50.6	100.7			19.2	
<i>Averages</i>									
	Serum	929.2±4.5	110.6 ±2.6	139.5±0.1	4.16±0.76	4.98±0.02		9.01±0.06	
	Change	+5.6	+1.8	-1.9	-0.50	+0.04		-0.83	
	Hemisphere	770.6±6.0	36.97±1.22	52.2±1.5	104.5±2.6	2.36±0.19	10.55±1.34	18.7±0.3	
	Change	+0.3	+0.26	+1.2	+8.9	+0.22	-0.71	-0.2	
	Cerebellum	752.4±9.9	36.54±1.67	51.8±1.9	100.0±3.5			19.5±0.2	
	Change	+7.4	+1.35	+1.0	+7.3			+0.4	
<i>C. Group II. Brain Removed 1.5 to 3.25 Hours after Trauma</i>									
7	Serum	931.2	116.0	133.9	3.83			8.20	1 1.25
	Hemisphere	763.4	37.75	41.8	99.1	1.30	10.82	19.3	
	Cerebellum	748.8	37.04	43.7	95.4			19.5	
6	Serum	917.8	104.2	133.8	3.50				2 2
	Hemisphere	765.6	34.77	48.4	101.5	1.56	10.92	19.4	1
	Cerebellum	743.1	32.66	51.4	102.4			20.0	
5	Serum	928.8	110.5	136.7	3.71	4.48		8.94	1 3
	Hemisphere	770.7	35.25	49.1	98.6	1.04	10.94	18.6	
	Cerebellum	751.2	34.99	51.8	92.0			19.7	
8	Serum	931.6	109.8	138.6	3.06	5.20		8.38	1 3.25
	Hemisphere	781.0	36.80	48.6	104.1	2.58	11.40	18.8	
	Cerebellum	761.0	35.20	50.9	104.1			19.2	
<i>Averages</i>									
	Serum	927.3±5.4	110.1±4.2	136.0±2.2	3.52±0.30			8.51±0.38	
	Change	+3.7	+1.3	-5.4	-1.14			-0.65	
	Hemisphere	770.2±7.1	36.14±1.19	47.0±3.0	100.8±2.2	1.67±0.56	11.02±0.22	19.0±0.3	
	Change	+10.7	-0.56	-4.0	+4.8	-0.47	-0.24	+0.1	
	Cerebellum	751.0±6.6	34.97±1.56	49.4±3.3	98.7±5.0			19.6±0.3	
	Change	+6.0	-0.22	-1.4	+6.0			+0.5	
<i>D. Group III. Brain Removed 5 Hours after Trauma</i>									
9	Serum	912.7	109.5	134.0	4.37	5.04	2.28	12.14	1 5.25
	Hemisphere	776.6	36.34	50.4	91.6	2.62	12.92	19.0	
	Cerebellum	758.9	36.46	54.8	100.0			19.5	
10	Serum	926.7	106.5	134.1	4.61			9.10	1 5.25
	Hemisphere	763.8	33.89	49.5	88.6	2.28	12.44	18.8	
	Cerebellum	748.4	33.76	50.4	98.0			19.2	

TABLE I. CONCLUDED

DOG NO.	TISSUE	H ₂ O	Cl	Na	K	Ca	Mg	TOTAL N	BLOWS	TIME AFTER BLOW
		gm/kg.	mEq/kg.	mEq/kg.	mEq/kg.	mEq/kg.	mEq/kg.	gm/kg		hr.
11	Serum	932.9	109.5	135.5	4.61	5.28		7.97	1	5
	Hemisphere	791.4	38.03	54.3	92.2	2.62	11.04	18.5		
	Cerebellum	748.8	34.11	55.2	97.4			20.2		
12	Serum	929.6	109.6	139.5	4.60	4.70		9.89	1	4.75
	Hemisphere	751.0	35.14	51.2	94.1	2.54	11.92	19.8		
	Cerebellum	737.0	34.74	51.8	95.5			19.7		
13	Serum	930.2	107.3	134.2	4.31	4.24		8.19	1	4.75
	Hemisphere	775.8	35.25	49.31	89.2	1.52	10.64	18.0		
	Cerebellum	759.3	33.58	48.95	89.4			18.8		
14	Serum	929.5	107.3	134.1	3.46	4.12		8.25	2	5
	Hemisphere	760.0	33.71	46.9	93.4	1.38	10.74	19.1		
	Cerebellum ¹	749.5	35.54	53.2	89.9			20.4		
	<i>Averages</i>									
	Serum	926.9±6.6	108.3±1.2	135.2±2.0	4.33±0.48	4.68±0.47		9.30±1.42		
	Change	+3.3	-0.5	-6.2	-0.33	-0.26		-0.54		
	Hemisphere	769.8±13.1	35.38±1.48	50.3±2.2	91.5±2.0	2.16±0.52	11.61±0.87	18.9±0.6		
	Change	+8.5	-1.33	-0.7	-4.1	+0.02	+0.65	0.0		
	Cerebellum	750.1±7.3	34.70±1.00	52.4±2.3	95.0±3.9			19.6±0.5		
	Change	+5.1	-0.49	+1.6	+2.3			+0.5		
<i>E. Group IV. Brain Removed 24 Hours after Trauma</i>										
15	Serum	921.1	105.5	126.7	3.59	4.72	2.40	9.80	1	24
	Hemisphere	764.3	33.95	48.25	100.2	1.20	9.48	19.0		
	Cerebellum	742.0	32.27	49.60	90.5			19.8		
16	Serum	925.7	98.8	125.4	3.88	4.38	1.84	9.26	1	24
	Hemisphere	762.2	34.73	45.13	100.2	1.20	9.92	18.4		
	Cerebellum	758.8	34.48	44.15	96.0			19.4		
17	Serum	921.6	110.0	139.0	3.98	4.58	2.92	9.50	1	24
	Hemisphere	757.3	32.78	50.4	102.7	1.40	9.44	19.9		
	Cerebellum	740.8	32.15	50.3	100.9			19.9		
	<i>Averages</i>									
	Serum	922.6±2.1	104.8±4.7	130.4±6.1	3.82±0.16	4.56±0.14	2.39±0.14	9.48±0.23		
	Change	-1.0	-4.0	-11.0	-0.84	-0.38	+0.49	-0.36		
	Hemisphere	761.3±3.1	33.82±0.53	47.9±1.8	101.1±1.0	1.27±0.09	9.61±0.21	19.1±0.6		
	Change	0.0	-2.89	-3.1	+5.5	-0.87	-1.65	+0.2		
	Cerebellum	747.2±8.1	32.97±1.07	48.0±2.8	95.8±4.3			19.7±0.2		
	Change	+1.2	-2.22	-2.8	+3.1			+0.6		
<i>F. Group V. Brain Removed 48 to 72 Hours after Trauma</i>										
18	Serum	922.4	106.6	137.4	4.68	4.54	2.48	9.60	1	72
	Hemisphere	776.0	36.33	49.4	104.8	2.30	11.70	19.2		
	Cerebellum	748.0	33.33	50.1	97.8			19.4		
19	Serum	926.9	109.8	138.2	5.59	4.26	2.26	8.80	1	48
	Hemisphere	764.9	35.80	48.95	103.8	2.90	12.00	19.0		
	Cerebellum	750.0	32.63	49.3	95.2			20.0		
20	Serum	919.0	108.4	138.8	4.42	4.36	1.72	10.06	1	72
	Hemisphere	761.0	35.80	53.8	99.9	2.32	11.72	19.2		
	Cerebellum	761.8	36.25	53.1	95.5			19.5		
	<i>Averages</i>									
	Serum	922.8±2.7	108.3±1.3	138.1±0.6	4.90±0.50	4.39±0.11	2.15±0.32	9.47±0.5		
	Change	-0.8	-0.5	-3.3	+0.24	-0.55	+0.25	-0.37		
	Hemisphere	767.3±5.3	35.98±0.25	50.7±1.8	99.8±6.4	2.51±0.28	11.81±0.14	19.1±0.1		
	Change	+6.0	-0.73	-0.3	+4.2	+0.37	+0.55	+0.2		
	Cerebellum	753.3±6.1	33.92±1.36	50.8±1.6	96.2±1.2			19.7±0.3		
	Change	+8.3	-1.27	0.0	+3.5			+0.6		

¹ Excess blood present in tissue.

phases or in both phases; *b*) the total water content of the tissue has not changed. Water has been transferred into the cells causing a decrease in the extracellular fluid phase and an increase in the intracellular fluid phase (intracellular edema), or water has been transferred from the cells causing an increase in the extracellular fluid phase (extracellular edema). In other words, only a change in the relative proportions of extra and intracellular phases has occurred.

Since the total water content of the hemispheres or cerebellum was not significantly increased in any of the groups of dogs studied, condition *a* does not have to be considered in this discussion. In no group of dogs was there found a greater increase in the total water content than 1.2 per cent (brains removed $\frac{1}{4}$ to $3\frac{1}{4}$ hr. after trauma). From table 1 the standard deviation in the control animals was 0.8 per cent. Therefore 1.2 per cent change cannot be considered a significant change. These findings agree with those of Pilcher (3, 13).

The extent to which a chemical approach can be made to the evaluation of edema in cerebral tissues is limited. Brain tissue unlike the skeletal muscle and liver is composed of many types of cells with variation in structure and function and reacts differently than the other tissues of the body. The generalizations concerning the evaluation of the extracellular phase or the intracellular phase in skeletal muscle and liver cannot be applied to brain tissue at this time. Further simultaneous chemical and histological studies are needed. Although quantitative interpretation of the analytical data for brain tissue into exact volumes of extra and intracellular phases cannot be made at this time, it is possible with some reservations to make tentative conjectures.

The concentration of ions in a tissue usually is indicative of the size of the phases in that tissue. Sodium and chloride are to be regarded as essentially extracellular materials in most tissues and are indicative of the size of that phase. Potassium and magnesium are to be regarded as essentially intracellular material in most tissues and are indicative of the size of that phase. The proportion of extracellular and intracellular phases varies from tissue to tissue but under normal conditions their proportions are relatively constant in any one tissue. For example, the extracellular phase volume/kg. of normal dog skeletal muscle averages 154 gm. \pm 27 gm. (9, 10). Therefore, the concentration of sodium and chloride in the normal hemisphere and cerebellum indicates a certain extracellular phase volume. The exact numerical size of this phase volume is not known at present, for it is believed that some of the sodium and chloride in brain tissue is intracellular (6).

If the concentrations of sodium and chloride of the normal brains are compared with the concentrations found in the brains following concussion, the only difference in concentrations will be found in the cerebral tissues removed 24 hours after the blow. The lowered sodium and chloride values in the tissues from this group were probably the result of the lowered sodium and chloride concentrations in the serum of this group. In other words, from all of the experiments reported here we have no evidence of any increases in the extracellular fluids in these cerebral tissues.

Since the total water content of the cerebral tissues did not change and indications are that the volume of the extracellular phase of the tissues did not change, the intracellular phase could not have changed. That the volume of the intracellular did not change is further suggested by the concentration of the intracellular ions

(magnesium and potassium) of the experimental cerebral tissues as compared to the concentrations in the control group.

The low calcium values found in the hemispheres of some of the dogs of *Group II*, *Group III* and all of the dogs in *Group IV* cannot be interpreted at this time. On the basis of dividing tissues into two phases, calcium is considered to be chiefly a constituent of the extracellular fluids of the body and, since there was no indication of a change in the volume of the extracellular fluid in the hemispheres of these experimental animals, calcium content should not have changed.

Eichelberger and McLean (11) reached the conclusion that unless some calcium is actually within the cells of tissues, a part of it must be assumed to be in an un-ionized combination with some extracellular substance other than the protein of the extracellular fluid, perhaps the connective tissue fibers. Further studies are needed for an interpretation of the low calcium values found here.

A further survey of the composite average changes shows that low sodium values in the serums prevailed in *Groups II*, *III* and significantly low values in *Group IV*. It seems, therefore, that salt has been abstracted without a change in the total serum water. This could be interpreted as meaning that salt was removed from the serums leaving this phase hypotonic. This change must be the result of a loss of sodium chloride probably caused by a disturbance in the function of the renal tubules. This is an instance of dehydration. This has been illustrated for the body as a whole by Darrow and Yannet's (12) experiments on the consequences of the intra-peritoneal injection of isotonic glucose. In view of these findings these animals of *Group IV* clearly would have been benefited by intravenous injection of isotonic NaCl. This might be a valuable key in the treatment of animals following concussion.

SUMMARY

Total water, nitrogen and electrolyte concentrations were determined in brains which were removed by bilateral craniotomy from dogs following pure concussion, uncomplicated by damage to blood vessels. The dogs studied were grouped according to the length of time allowed to elapse between the impact of the blows and the removal of the brain in order to attempt to determine the time required for post traumatic cerebral changes.

There was no indication of cerebral swelling in these experiments. The total water content of the hemispheres or the cerebellum did not change significantly. Neither was there any indication of the presence of a redistribution of water in the cerebral tissues; that is, there was no shifting of water from the extracellular phase to the cell phase (intracellular edema) or vice versa (extracellular edema).

Low calcium values were found in the hemispheres of some of the dogs killed at $1\frac{1}{2}$ hours (*Group II*) and 5 hours (*Group III*) and all of the 24-hour group. The causes are discussed but cannot be interpreted at this time. There was a low sodium concentration in the serums of the dogs, 24 hours after trauma. This finding may have implications in the consideration of therapy for post traumatic cerebral injury.

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CONDITIONS MODIFYING RESISTANCE TO ACCELERATORY FORCES AND PROTECTION BY ABDOMINAL PRESSURE¹

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SEVERAL factors are believed to affect the resistance of the organism to acceleratory forces. Some of these have been made the subject of earlier inquiry (1); altered reactions which are dependent upon common bodily disturbances call for more extended consideration, however, in view especially of hazards inherent in man's greatly speeded movement through space in modern times. Moreover the acceleratory forces involved in everyday aircraft (especially military) operation differ only in intensity from that of gravitation, ever imposed on us and producing not only its stimulus but a formidable train of stigmata which call for clearer understanding. Under controlled conditions modified responses during acceleratory exposure only two or three times that of gravity have now been investigated in a large group of general and specific body states induced by activity, dietary, thermal and other procedures. Protective measures against reduced levels of resistance to acceleration and what may be termed the functional cost of various disturbances are also considered.

METHODS

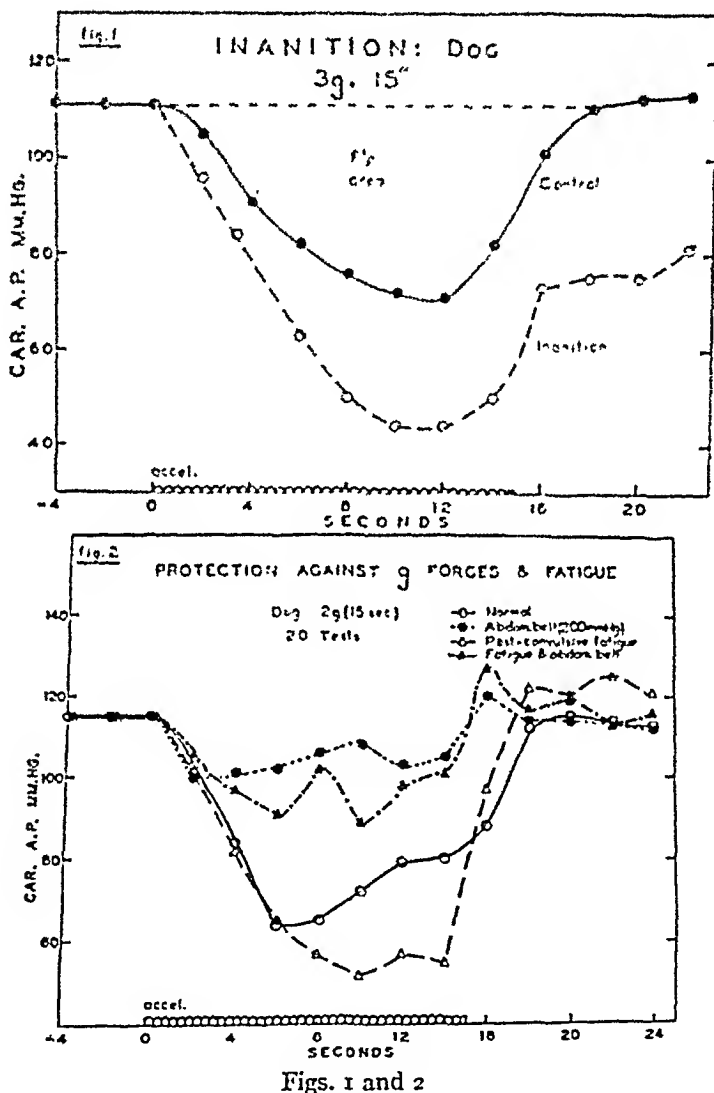
Dogs were used in the 20-hour fasted state. Minor surgical work was carried out under light ether and novocain; no anesthetic was used during tests except as

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noted later. The right carotid artery was opened for determining arterial pressure, heparin injected and a through T-cannula allowing near-normal cerebral circulation introduced. An electrometric method registering blood pressure continuously was used (2).

The carotid arterial pressure changes evoked by acceleratory exposure were used as criteria. Specifically a 'circulatory area', bounded by the arterial pressure depression curve and the connecting horizontal line above, and designated 'pt_p' (fig. 1),



Figs. 1 and 2

was utilized in evaluating responses as described previously (3). This gave the most faithful measure of the magnitude of the involved vascular change under acceleration. Also the overall change in time given by this circulatory area indicated more accurately the blood-vascular changes under stress in the cerebral tissues.

Exposures to acceleration were made on a centrifuge with a 10-ft. rotor arm. Only positive g forces were employed. From 5 to 10 normal runs at 3 g (or occasionally 2 g in case of a poorly-resistant animal) for 15 seconds were first given with rest

intervals of 2 to 3 minutes between runs. This amount of exposure was relatively light, and animals were found in good condition after a large number of tests given over a 4 to 6 hour experimental period. One animal for example showed no reduction from its normal response after 50 control tests in 2 hours. Tolerance to g forces could be lowered rather quickly, however, if much briefer intervals between runs or severe or prolonged exposures were given. Such conditions were avoided in the present work and uniform control observations were followed over long periods. Between transient-induced body states, e.g. under adrenalin, narcotics, etc., control runs could thus be made rather frequently.

Results of initial and later interspersed control runs served as normals or basals for comparison with concurrent or subsequent experimental tests. Each animal served as its own control, therefore, except in the special cases of inanition and adiposity, in which comparison was made with the carotid arterial pressure average of all normal tests (over 450 cases). In several cases in which the more severe reductions in resistance to g were observed, tests were made of the efficacy of a pneumatic cuff or belt placed around the pelvic or lower abdomen and inflated to 150 mm. Hg.

A rather large number of tests were made in most of the experimental series; in 30 different groups there were 535 tests, or an average of about 18 tests per group. Experiments were carried out on animals showing a mean carotid arterial pressure of 110 to 140 mm. Hg. Excluding actual centrifugal exposures, the experimental procedures usually produced slight (within 10 mm. Hg) or no change in the basal resting level. When the base carotid arterial pressure level fell to about 100 mm. Hg., usually in 5 to 6 hours, tests were discontinued. Further points on technique follow.

Fatigue. After normal runs had been taken, from one to three series of vigorous convulsive reactions were evoked by metrazol given intravenously (usually 2 cc/8-kg. dog) to produce fatigue. After convulsions had ceased, several centrifuge tests were made within 20 to 30 minutes; following this, recovery of normal response commonly occurred.

Trauma. Under light anesthesia *a*) the abdominal wall was given several taps (6-8) or *b*) a small incision was made and the intestinal coils manipulated, to produce a temporary shock-like condition in each case. Reduction of the base arterial pressure by about 10 mm. Hg indicated adequacy of the trauma, and runs were made over 10 to 20 minutes, usually before recovery.

Heat; cold. Hot water or crushed ice in rubber bags was applied to abdominal and chest areas; occasionally also, a slow injection of warm or cold saline was given intravenously. Rectal temperature shifts of 1 to 2°C. were produced.

Inanition; adiposity. Animals were kept on either low or high caloric intake for three to six weeks to induce respectively significant loss or gain of weight. In these cases only were the arterial pressure reactions compared with the average derived from all the normal series.

Adrenalin. An intravascular dose given slowly and producing usually 10 to 20 mm. Hg rise in basal carotid arterial pressure was employed. Aqueous and oil preparations were utilized, and tests were made usually within the following 1 to 10 minutes.

Hypertonus (Metrazol); Struggling. Small doses of metrazol (0.25 cc/8-kg. dog)

were given until slight muscle tremors, usually of the limb extremities, set in. Struggle records used were those which appeared spontaneously in the group of animals studied.

Narcosis. Ether; nembutal and pentothal were given to induce fairly deep narcosis, and reactions to acceleration observed usually for 15 to 30 minutes thereafter.

Alcohol. Ethyl alcohol was given intravascularly in weak solution (10-20%) to induce blood alcohol levels of 0.10 to 0.25 (calculated). General condition of the animal also was followed.

CO₂; tobacco smoke; asphyxia. CO₂ was administered intratracheally for various periods (usually 10-90 sec.) in various concentrations (CO₂ up to 15%) to produce slight to severe degrees of hyperpnea. Tobacco smoke was given similarly by burning cigarette tobacco in a chamber. Asphyxia was produced by clamping the tracheal tube for 30 to 90 seconds to induce the desired degree of respiratory disturbance.

Pelvic belt. A sphygmomanometer with wide inflatable cuff or belt to cover the animal's lower abdominal wall was utilized. It was kept in place from the start of an experiment, and inflated and deflated respectively a few seconds before and after actual exposures to acceleration.

RESULTS

Throughout a series of 50 or more standard control exposures to an acceleratory force of 3 g for 15 seconds each, within about 2 hours, most dogs showed strikingly similar reductions in carotid arterial pressure. Tables 1 and 2 summarize the main experimental results, and figures 1 to 3 show typical and diagrammatic curves. Positive (+) and negative (−) marks in the tables indicate greater or less depression of carotid arterial pressure in the experimental test compared to the normal decrease in the untreated control run at the same g force. It is apparent, therefore, that reduced and increased tolerance respectively are indicated by + and − signs.

A rather large number of conditions are observed to reduce resistance to acceleration; in many cases the reductions reached, indeed, about 50 per cent—in fatigue, trauma, inanition, adiposity, etc. In the 15 groups totaling 310 cases observed in which there appeared decreased tolerance to g (*series A*, table 1), the average disability was 35 per cent, with a range of 13 to 74 per cent. The limits (time, degree) to which the condition was imposed were obviously contributory factors in the response. While the degree of the induced disturbance was not specifically determinable in some cases, it did not in any instance reach an extreme, and animals showed return to the normal (control) response to g usually in 5 to 15 minutes.

Of the relatively few conditions which increase resistance to acceleratory forces, the application of pressure in the pneumatic pelvic cuff was by far the most efficacious; it was about twice as good as the four next best conditions. In some cases protection was almost complete against 3 g, 15 seconds with pelvic pressure, only slight reduction in carotid arterial pressure occurring throughout exposure. Those conditions which definitely involved muscular hypertonus—struggling, body tremors, cold, CO₂ treatment—also stood out rather markedly; much smaller reductions in

carotid arterial pressure under g, about 30 to 40 per cent less than in the controls, were observed in these cases (table 1).

TABLE 1. RESISTANCE TO ACCELERATION UNDER VARIOUS CONDITIONS
Determined by area changes (pt_p) in carotid arterial pressure. Dogs: 3 g, 15 sec.¹

SERIES A: CONDITIONS WHICH DECREASE TOLERANCE TO G	NO. OF TESTS	DIFFERENCE BETWEEN NORMAL AND DISTURBED ARTERIAL PRESSURE DECREASE	SERIES B: CONDITIONS WHICH INCREASE TOLERANCE TO G	NO. OF TESTS	DIFFERENCE BETWEEN NORMAL AND DISTURBED ARTERIAL PRESSURE DECREASE
		pt_p %			pt_p %
Fatigue (after metrazol convulsions).....	22	+43	Pelvic belt applied.....	15	-72
Trauma			Vigorous struggling.....	10	-38
Abdominal wall.....	47	+51	Muscular tremors (after met-		
Intestinal.....	10	+29	razol).....	22	-41
Heat applied.....	20	+14	Cold applied.....	14	-28
Inanition (after fasting).....	16	+56	Adrenalin injected.....	19	-12
Adiposity (overfeeding).....	35	+52	Partial anoxia, etc.		
Anoxia, etc.			Asphyxia, light.....	12	-16
Asphyxia—moderate.....	4	+22	CO ₂ , light.....	13	-39
CO ₂ , moderate.....	8	+19	Tobacco smoke, light....	15	-2
severe.....	11	+74	Alcoholism, light dosage	45	-17
Tobacco smoke					
severe.....	14	+31			
Narcosis					
Ether.....	20	+41			
Pentothal.....	38	+15			
Nembutal.....	20	+31			
Alcoholism					
moderate dosage.....	18	+13			
heavy dosage.....	27	+31			

¹ In a few cases 2 g, 15 sec., was applied.

TABLE 2. PROTECTION AGAINST G BY PELVIC PRESSURE
Dogs: 3 g, 15 sec.

CONDITIONS	DIFFERENCE BETWEEN NORMAL AND DISTURBED CAROTID ARTERIAL PRESSURE DECREASE			
	Pelvic belt pressure off		Pelvic belt pressure on	
	No. of Cases	pt_p %	No. of Cases	pt_p %
Fatigue (after convulsions).....	22	+43	12	-37
Trauma (abdominal blows).....	47	+51	11	-41
Asphyxia (moderate).....	4	+22	9	-60
Alcoholism (heavy dosage).....	27	+31	3	-59
Narcosis (ether).....	20	+41	10	-45
Pelvic belt applied.....			15	-72

While a slight advantage found after adrenalin injection was expected from earlier work, the apparent benefit from light alcohol dosage was surprising. Lowered

tolerance to g forces under heavier alcohol treatment was nevertheless consistently observed. Similarly under certain anoxic or hypercapnic types of interference with the normal animal economy, greater resistance was found under light treatment, giving way to marked handicap under g stresses when the conditions were pushed to greater limits.

Moderate to heavy doses of amyl nitrite did not alter significantly from the controls the carotid arterial pressure curve elicited by acceleration. Basal arterial pressure levels in these cases, however, were reduced by 10 to 20 mm.Hg.

Small amounts of hemorrhage, up to 5 cc., did not affect responses to g forces; removal of 10 to 30 cc. of blood from an 8-kg. dog reduced tolerance, however, as shown by the greater than normal fall in carotid pressure on centrifugation. Subsequent transfusion of plasma or serum was doubtfully helpful in a few tests.

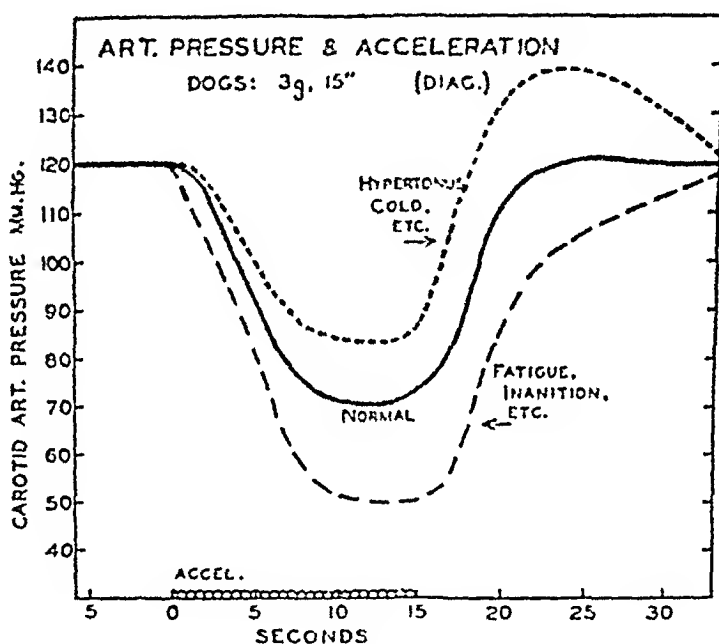


Fig. 3

The introduction of pressure into a pneumatic system covering the pelvic area was strikingly protective against serious fall in carotid arterial pressure during acceleration in many types of traumatic and deteriorating conditions. Invariably productive by themselves of severe handicaps under g stresses, these conditions were controlled almost completely by a cuff or belt pressure of 150 mm.Hg. In the whole group of conditions studied, a reduced tolerance of 38 per cent without protection was converted into an increased resistance of 63 per cent above the normal control level with the abdominal belt inflated (table 2).

DISCUSSION

Use of a rather moderate acceleratory force in these experiments was calculated to indicate more clearly the possible functional advantage or disadvantage of certain common body states with respect to the applied force. Correlation of the results obtained with reactions of the organism to gravitational influence also would be more

pertinent. A stress of three times gravity applied through unit time ($3 g \times 1 t$), as used herein, might not for example differ greatly in functional influence from $1 g \times 3 t$. In many instances at least it has been shown that within limits the product of g and t (force-time) represents the responsible influential factor (1-3).

Characteristic of the depressed responses shown by the larger group of experimentally treated animals to even the moderate acceleratory forces used (*series A*, table 1) was the delayed return of normal carotid arterial pressure after the centrifuge had stopped. In the fatigued, fasted and other groups, from 20 to 60 seconds elapsed before recovery. Often the actual level to which arterial pressure fell in these cases, however, was only 10 to 20 mm.Hg. greater than in the normal controls.

In those tests in which animals showed greater resistance to g exposure, a sharp rebound phenomenon was a common feature of the arterial pressure curve. In both the hypertonic and the cooled groups, for example, a quick post-acceleratory rise of 10 to 30 mm. Hg in carotid pressure level marked the reaction curve. There was also a comparatively smaller overall arterial pressure reduction evident throughout these tests (fig. 3).

A number of the conditions considered above are similar to those practical operational influences which are experienced during flight in the plane. Military activities particularly may involve exposure to fatigue, trauma, hemorrhage, anoxia, ambient thermal changes etc., and it is clearly important to understand the effects of such variables on resistance or susceptibility to rather commonly experienced acceleratory forces. Readily evident is the pronounced disability produced especially by such factors as the above, as well as inanition and overfeeding, narcotics and alcohol in excess.

It is unfortunate that there is such a paucity of factors which alleviate the severity of the arterial responses, in the functional test herein used. The alleviatory factors too are usually vicariously provided rather than spontaneously developed. By analogy, under gravitational influences as under g , protective conditions would surely be few in number. That certain adjustments of the body physiologic may be effected to provide 1 to 2 g advantage, as herein demonstrated (e.g. in struggling), does nevertheless afford some comfort. Importance may be attached, in this connection, to a self-protective manoeuvre described by Wood and his colleagues (4). Such reinforced arterial pressure responses under acceleration are probably explicable on the basis of altered muscular and vasomotor conditions. Vasoconstrictor responses and later carotid sinus activities must be considered as involved.

The results show that fairly high-grade mammals are able to resist rather poorly a force about three times that of gravity applied for 10 to 15 seconds only. Man apparently does much better than sub-primates under this contingency (3); travail through long ages, ever fighting gravity along with his sanguinary struggles, appears to have resulted in a fair degree of acclimatization.

It is recognized, however, that resistance to the force of gravity varies considerably in different individuals, and in the same person at different times. Some show syncopal attacks readily, or suffer various ptoses, or varicosities; rather frequently under stressful conditions, in older age groups and others, a position of recumbency becomes desirable or necessitous. While as human beings we have achieved upright-

ness, it is clear, we do not retain the vertical position too well, and now and then do better functionally to 'take it' lying down!

These disturbed body conditions, inadequacies and demands for rest indicate significantly the functional cost of man's continual exposure to gravitational pull. This ever-acting force, as in the case of applied acceleration, exerts its action on body fluids and visceral organs particularly. Admitted that on the whole there has accrued and is yet accruing a tremendous functional credit balance—of manual and head (most importantly cerebral) freedoms—through our achievement of verticality, reflexes of supply and control still show considerable lack of competence in the sub-par individual, and more rugged organisms also pay their part toward orthograde progress.

In examining the responses in a comprehensive group of common body states such as those noted above, it is clear that a significant, aggravative reduction in the main arterial blood supply to the head, more than 50% beyond control levels in many instances, takes place under positive acceleratory stresses. An increased knowledge of such sensitive reactions as those now considered should lead to a better understanding of man's age-long responses to the influence of gravitational force.

SUMMARY

In a number of commonly experienced bodily conditions responses to moderate acceleratory forces (2-3 g, 15 sec.) were considerably modified, as shown by carotid arterial pressure changes in the dog. Fatigue and trauma, inanition and adiposity decreased markedly the tolerance to g; usually the arterial pressure reductions in these cases were 50 per cent greater than in the controls. In certain cases of anoxia, and following administration of narcotics and ethyl alcohol, resistance to acceleration was also greatly reduced.

Spontaneous bursts of struggling, muscular tremors after metrazol treatment, and also breathing of CO₂ in small amounts resulted in definite improvement in tolerance; about 1 g advantage was effected in these cases. The application of cold to the body also was only slightly less advantageous. Tobacco smoke and alcohol given in small amounts were definitely not deleterious by the carotid arterial pressure criterion; in the tests with light doses of alcohol, indeed, significant benefit appeared to be conferred. Application of a lower abdominal or pelvic belt inflated to 150 mm. Hg pressure gave by far the greatest protection against g stresses. Even after severely depressing conditions of fatigue, trauma, etc., had been imposed upon the animal, protection was well maintained. In these cases an advantage of 2 to 3 g was commonly observed.

Correlation of tests on applied acceleration with the effects of gravitational force on the body is discussed.

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RESUSCITATION FROM OBSTRUCTIVE ASPHYXIA¹

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IN A previous paper (1) the results of a comparative study of the relative effectiveness of various gases and methods of artificial respiration for resuscitation from carbon monoxide asphyxia in dogs was reported. A significant difference in the incidence of immediate survival and post-asphyxial neurological sequelae was not found when 100 per cent oxygen² or 7 per cent carbogen (7% CO₂ and 93% O₂) was used as the gas for resuscitation. Mechanical resuscitation methods were found to be superior to the manual method because greater pulmonary ventilation was obtained with the former.

This work was undertaken to determine if a difference might exist in the effectiveness of different oxygen containing gases and methods of artificial respiration for resuscitation from obstructive asphyxia. It was believed that obstructive asphyxia, for various reasons which need not be mentioned here, might be different from carbon monoxide asphyxia as regards response to resuscitation procedures. For this reason the experiments recorded in this paper were deemed necessary.

EXPERIMENTAL

Determination of the Stage of Asphyxia (End-Point) at Which to Start Resuscitation. In our previous study (1) it had been shown by the study of a control group of dogs that the least variable and most propitious time to start resuscitation was on the manifestation of the first gasp or evidence of terminal air hunger. The need for the establishment of such an 'end-point' had been overlooked by previous investigators in this field, but our data showed that it is necessary for any comparative study on resuscitation.

The first step in this study, then, concerned the establishment of the best 'end-point' or stage of obstruction asphyxia at which to start resuscitation.

In a group of experiments on 12 dogs pentobarbital anesthesia was used, and the femoral artery cannulated for a blood pressure record, a pneumograph attached to the chest, and the trachea cannulated so that it could be obstructed precisely by the insertion of a stopper at the end of inspiration. The time of disappearance of heart sounds was recorded by auscultation with a stethoscope. When all the changes on the kymograms were analyzed, the time of their occurrence was so varied that a reliable 'end-point' could not have been arrived at without using some 50 animals.

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² Provided by The Linde Air Products Company.

It was believed that the variable results were due to the use of a general anesthetic, the depth of the anesthesia not being readily subject to control. Accordingly, local anesthesia (1% procaine) was used in all subsequent experiments.

Under local anesthesia 19 dogs were obstructed and the time at which various changes occurred were recorded. The mean time at which the various changes occurred are shown in figure 1 and the ranges in table 1. In each instance the resulting histogram was quite 'normal' in contour. Consciousness was lost quite promptly

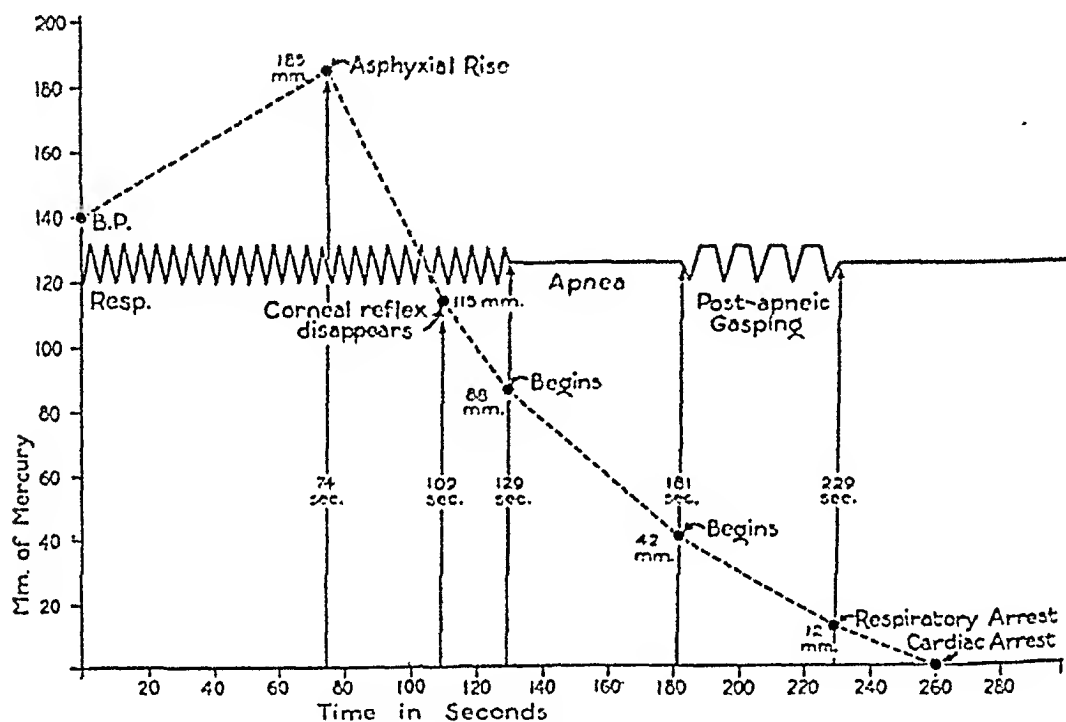


Fig. 1

TABLE I

TIME FROM OBSTRUCTION TO	AVE. Sec.	RANGE Sec.	TIME FROM OBSTRUCTION TO	AVE. Sec.	RANGE Sec.
Loss of corneal reflex	109	60-180	Respiratory arrest	230	100-340
First onset of apneic period	129	60-220	Cardiac arrest	259	140-380
First onset of post-apneic gasp	181	100-300	Time from first gasp to respiratory arrest	48	5-104

and the corneal reflex was lost on an average of 1.75 minutes. Apnea, variable in character, frequently complete, started and was then interrupted by a series of post-apneic or terminal gasps; without exception the heart continued to beat after the cessation of respiration (fig. 1 and table 1).

Because the first terminal gasp was used as an 'end-point' for starting resuscitation in the experiments in carbon monoxide asphyxia, it was thought that this might be the best point at which to start resuscitation in these experiments. Since it was less troublesome to observe the blood pressure level of 42 mm. Hg and because it coincided on the average with the occurrence of the first terminal gasp, it was decided

to use such an 'end-point'. So in all subsequent experiments the tracheal obstruction was removed when the blood pressure reached 42 mm. Hg.

Results of Resuscitation Using Various Procedures. A total of 103 dogs were divided into five groups. In a *control group* of 22 dogs, the tracheal obstruction was removed and no other treatment was given; 32 per cent survived. *Manual artificial* respiration in air was given to a second group of 20 dogs; 35 per cent survived. *Mechanical artificial*³ respiration was given to the next three groups but air was the gas used in one group, 100 per cent oxygen was used in the next and 7 per cent

TABLE 2. RESULTS OF RESUSCITATION FROM OBSTRUCTIVE ASPHYXIA

TREATMENT		BLOOD PRESSURE, MM. HG		DURATION OF OBSTR.	TIME OF ONSET OF 1ST. GASP AFTER ³ RESUS.	BLOOD PRESSURE AT FIRST GASP	NUMBER OF DOGS		
		Before Obstr.	After Obstr. ²				Lived	Died	% Living
None	All dogs	149	155	sec.	sec.	mm. Hg	7	15	32
				204					
	Survivors only			187	19	33			
Manual artif. respiration with air	All dogs	143	193	222			7	13	35
				213	42	48			
Mechanical ¹ artif. Respiration with air	All dogs	125	153	195			15	5	75
				193	42	136			
Mechanical ¹ artif. with 100% O ₂	All dogs	126	170	179			15	6	71
				199	38	101			
Mechanical ¹ artif. with 7% carbogen	All dogs	138	174	124			17	3	85
				128	53	110			

¹ Alternating positive and negative pressure. ² Asphyxial rise. ³ Time of onset of first spontaneous gasp after relieving obstruction and indicating survival.

carbogen in the last. The percentage of animals surviving was 75, 71, and 85 per cent respectively (table 2).

The difference between 32 and 35 per cent survival on the one hand and 75, 71, and 85 per cent on the other is statistically significant. The value of 85 per cent survival, using 7 per cent carbogen, is not significantly greater than 75 or 71 per cent, since it could readily occur by chance.

DISCUSSION

The significantly larger percentage of survivals with mechanical artificial respiration is obviously due to the fact that a greater minute volume of air flow was ob-

³ A positive and negative type of resuscitator was used.

tained with mechanical than with manual respiration. This emphasizes the importance of getting fully oxygen-saturated blood to the heart, failing from anoxia, as quickly as possible.

It is of interest to point out (table 2) that although the time of onset of the first spontaneous gasp after the administration of manual or mechanical respiration was approximately the same, the blood pressure at the time of the onset of the first gasp was much higher when mechanical respiration was given. This might be explained by the mechanical massaging action of alternating positive and negative pressure on the heart and pulmonary circulation, as might be suggested by the observations of Thompson and his colleagues (2-4). However, such an observation is more likely due to the same factor which increased the number of survivals, namely, the delivery of a better and quicker supply of oxygen to the failing heart.

SUMMARY

Obstruction asphyxia was produced in 19 dogs to establish the range and mean values in the time of onset of the critical changes which follow such a procedure. It was found that the occurrence of the first terminal gasp which had been used as the point for starting resuscitation in our experiments on carbon monoxide asphyxia corresponded with a mean blood pressure of 42 mm. Hg. This blood pressure value was taken at the point at which resuscitation would be applied to obtain comparative values with different methods for resuscitation. The following observations were made. Thirty-two per cent of 22 dogs survived when no treatment was given. Thirty-five per cent of 20 dogs given manual artificial respiration in air survived. Seventy-five per cent of 20 dogs survived when mechanical artificial respiration in air was used. Seventy-one per cent of 21 dogs survived when mechanical artificial respiration with 100 per cent oxygen was used. Eighty-five per cent of 20 dogs survived when mechanical artificial respiration with a mixture of 7 per cent CO₂ and 93 per cent O₂ was used. The mechanical artificial respiration was more effective in producing a large minute volume of ventilation than manual respiration; this accounts for its greater effectiveness in resuscitation. The type of gas used produced no significant difference in the results.

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CEREBRAL CONSTITUENTS IN RELATION TO BLOOD GASES

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THE effects of hypoxia on the cerebral lactic acid and acid-soluble phosphorus compounds were described in a previous paper from this laboratory (1). It was shown that in the dog the cerebral lactic acid level begins to increase when the oxygen saturation of the cerebral venous blood is reduced below a critical level of 28 to 43 per cent, and that phosphocreatine breakdown in the brain becomes measurable when the cerebral venous blood oxygen saturation is reduced below 15 to 22 per cent.

The hypoxia induced in these experiments was associated with hyperventilation and acapnia. Gibbs, *et al.* (2) have maintained that the electroencephalographic changes induced by hypoxia (breathing 6-8% oxygen) are related primarily to the decrease in the cerebral carbon dioxide tension which occurs. It therefore appeared desirable to study the influence of carbon dioxide on the chemical changes occurring in the brain during hypoxia.

In the present investigation the calculation of the oxygen and carbon dioxide tensions of the arterial and cerebral venous blood has been made possible by including measurements of blood pH in addition to the gasometric analyses. Determinations of cerebral glucose and glycogen have also been included in this series.

Methods of the type employed here have been used in studies of chemical changes in the brain associated with convulsive activity (3, 4). In such experiments it is usually necessary to inject a paralyzing drug and to maintain the animal on artificial respiration. Under these conditions variations in the blood carbon dioxide and oxygen are likely to occur. It was therefore considered important to investigate the possible effects of such variations on the chemical pattern of the cortex, and thus to define the permissible limits of variation of the blood gases in studies of the effects of convulsants or other agents. The results indicate that the variations in carbon dioxide tension which are likely to be encountered in such experiments are not a disturbing influence. The oxygen tension of the cerebral venous blood should be maintained above 25 mm. Hg; variations above this level appear to have no effect on the cerebral lactic acid or other constituents studied.

METHODS

The dog was given 20 mg. morphine sulfate per kg. body weight by subcutaneous injection. A Magill intratracheal tube with a Waters-Guedel inflatable cuff was inserted. Local infiltration

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with 0.25 per cent procaine hydrochloride was used for exposure of the skull and of the femoral arteries. A trephine opening was made over the superior sagittal sinus and the bone of the calvarium was removed over a wide area, leaving the dura intact. One femoral artery was cannulated for the measurement of blood pressure.

Mixtures of oxygen, carbon dioxide and nitrogen were given from a 5-liter breathing bag connected to a Foregger anesthesia apparatus. During the period of administration of the desired mixture, 12 to 14 minutes after its inception, blood specimens were drawn simultaneously from a femoral artery and from the sagittal sinus. The blood was collected anaerobically over mercury, with heparin as the anticoagulant. The brain was then frozen *in situ* with liquid air,² exactly 15 minutes after the administration of the gas mixture was begun. The specimens taken for analysis included the accessible areas of the cortex of both hemispheres to a depth of approximately 1 cm.

In certain experiments the arterial carbon dioxide tension was decreased by hyperventilation with air or oxygen. The animals of this group were immobilized by intravenous injection of a 0.75 per cent solution of dihydro-beta-erythroidine hydrobromide³ in physiological saline (1 cc/kg. body weight), the dose being sufficient to induce respiratory paralysis. Artificial respiration at the desired rate and volume was then given by means of a respiratory pump. Blood specimens were obtained after 12 to 14 minutes, and the brain was frozen after 15 minutes of hyperventilation. In this group of experiments the electrocorticogram was recorded, from monopolar leads in the parieto-occipital region.

Blood Gases and pH. The oxygen content and capacity and the carbon dioxide content of the blood were determined by the manometric methods of Van Slyke and Neill (5). Blood pH was determined anaerobically at 38° C. within a few minutes after the specimen was drawn by means of a glass electrode and a voltmeter similar to that described by Nims (6). The standard buffers used were those of Hastings and Sendroy (7), since it was desired to calculate the carbon dioxide tension from the line charts of Van Slyke and Sendroy (8) based on the same pH scale. Root *et al.* (9) have shown that these charts are applicable to dog blood. Oxygen tension was calculated from the oxyhemoglobin dissociation curve of blood of normal men at pH 7.40, the appropriate pH corrections being applied (10, 11). The oxyhemoglobin dissociation curve of dog blood does not differ significantly from that of human blood (12). It is recognized that the calculated values of oxygen tension at the upper end of the dissociation curve (above 85% saturation) are subject to certain errors and tend to be slightly low (13). However, the values in this upper range are not critical in these experiments. The method is not applicable for values of oxygen tension above 150 mm. Hg, and such values are recorded as '150+'.

Cerebral Constituents. Glucose was determined on cadmium hydroxide filtrates of brain (and of blood) by reduction of an alkaline ferricyanide reagent and titration with ceric sulfate electrometrically. Traces of cadmium were removed from the filtrates by precipitation with disodium phosphate. It was found necessary to correct for non-sugar reducing substances, determined after fermentation by washed yeast cells. Glycogen was determined by the procedure of Kerr (14), with substitution of the ferricyanide reduction for the copper reduction. Here the glucose reduction factor was corrected for the presence of excess sodium chloride in the neutralized hydrolysate of glycogen. Lactic acid was determined by a modification of the method of Barker and Summerson (15). Inorganic phosphate, phosphocreatine and adenosine triphosphate were determined by methods previously described (16).

² Kindly supplied by the Wall Chemicals Division of the Liquid Carbonic Corporation, Detroit.

³ Courtesy of Merck and Company, Inc., Rahway, N. J.

RESULTS

Morphinized Dogs Breathing Air. Only three control animals have been included in this series, since a large group has previously been published (17) establishing the following average normal levels of cerebral constituents: lactic acid, 12.1 mg/100 gm.; inorganic phosphate, 7.8 mg. P/100 gm.; phosphocreatine, 9.1 mg. P/100 gm.; adenosine triphosphate, 19.1 mg. acid-labile P/100 gm. (representing two phosphate groups) and 9.8 mg. ribose monophosphate P/100 gm. (representing the third phosphate group). It was also shown that the paralyzing drug dihydro-beta-erythroidine hydrobromide as used in some of these experiments causes no significant changes in the above-mentioned constituents.

The normal glycogen content of the dog's brain has been established by Kerr and Ghantus (18), who found in a series of 22 animals values of 77 to 150 mg/100 gm. with an average of 108 mg/100 gm. The values reported in table 1 are in this range, with the exception of one low value occurring in severe hypoxia.

As shown by Kerr and Ghantus (18), the glucose content of cerebral tissue varies with the blood glucose but is normally below the level in the blood. The values re-

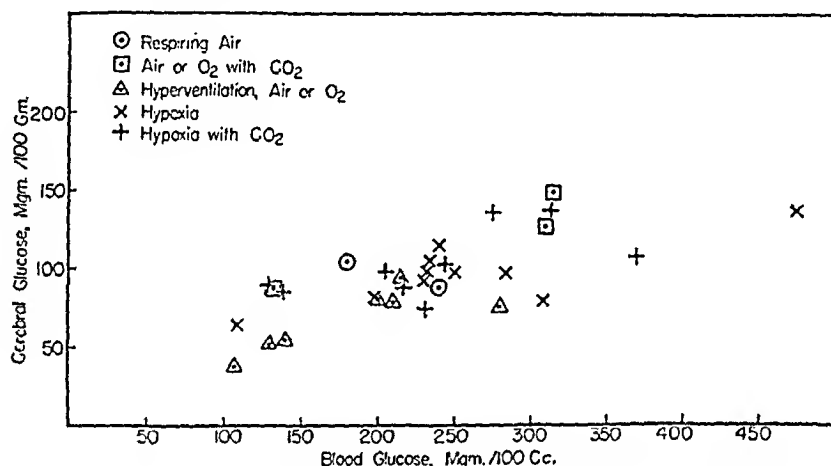


Fig. 1. RELATION of cerebral glucose to arterial blood glucose

ported here are in agreement with their findings (fig. 1). The blood levels are elevated by the action of morphine (19) and in some instances by hypoxia.

Variations of Arterial Carbon Dioxide Tension in the Presence of Adequate Oxygen. Addition of 5.5 to 9 per cent carbon dioxide to air or oxygen increased the arterial carbon dioxide tension but brought about no significant changes in the cerebral constituents under investigation (table 1).

Hyperventilation with air or oxygen greatly decreased the arterial carbon dioxide tension. Although the oxygen tension of the cerebral venous blood was decreased to the verge of the hypoxic level, due apparently to cerebral-vasoconstriction, the cerebral constituents showed no significant changes except that in four instances the lactic acid level was increased. This change is to be ascribed to oxygen deficiency rather than to the decreased carbon dioxide tension, since in three of these animals the blood pressure fell when the final blood samples were drawn and just before the brain was frozen; in the fourth instance (in which lactic acid was only slightly increased) the cerebral-vasoconstriction was so extreme that a sufficient amount of blood for analysis could not be obtained from the sagittal sinus.

TABLE 1. CEREBRAL CONSTITUENTS IN RELATION TO BLOOD GASES

Dog	ARTERIAL BLOOD					CEREBRAL TISSUE ¹					
	O ₂ tens.	CO ₂ tens.	Change in CO ₂ tension ²	Lactic acid	glucose	Venous blood O ₂ tens.	Lactic acid	Glucose	Glyco-gen	Inorg. P	Phos-pho-creatine
	mm. Hg	mm. Hg	mm. Hg	mg/100 cc.		mm. Hg		mg/100 gm.		mg. P/100 Gm.	
<i>Breathing Air</i>											
1	61	44				37	13.8	115	94		
2	63	58			180	47	9.1	104	102	8.7	8.8
3	53	49		11.1	240	32	13.8	88	123		8.1
<i>Breathing Air with 5.5 to 9% CO₂</i>											
4	76	70	+9	17.7	310	55	7.7	128	116	7.6	10.0
5				21.4	119		7.5			8.6	8.8
6	65	88	+28	31.9	133	50	8.5	87	118	9.0	8.9
<i>Breathing O₂ with 5.5% CO₂</i>											
7	150+	73	+17	10.7	315	61	6.8	149	106	6.1	9.8
<i>Breathing Mixtures Low in Oxygen, with Acapnia</i>											
8	35	30	-18	30.9	283	17	23.0	97		10.4	6.4
9	33	47	-14	50.0	474	17	22.7	137			8.3
10	22	45	-14	32.7	202	16	28.4			8.1	8.9
11	21	40	-30	63.0	240	14	39	115	140	8.6	9.6
12	16	33	-10	38.5	230	10	41	92		10.8	7.3
13	11	40	-8	65.6	271	8	94			12.3	4.4
14	28	30	-5	41.8	109	7	50	64		10.4	5.8
15	11	29	-21	59.0	198	7	90	81	106	12.0	4.2
16	9	35	-17	40.6	232	4	85	98	83	12.4	4.3
17	3	19	-24		250	3	208	97		19.6	0.7
18	9	18	-20	105.0	234	2	104	105	101	11.8	4.8
19	6	37	-17	143.0	308	0	194	79	57	19.9	0.5
<i>Breathing Mixtures Low in Oxygen with 5 to 6% CO₂</i>											
20	23	52	0	23.4	139	17	19.6	85	144		
21	20	52	-2	19.6	217	15	16.4	93	122	8.9	8.6
22	18	66	+6	24.7	205	13	33.5	98	116	9.8	6.4
23	18	68	+16	31.6	275	13	18.9	136		9.5	7.0
24	15	48	-2	38.3	244	9	30.0	103	131	9.3	7.2
25	15	48	-8	67.0	129	6	25.6	89	142	9.6	8.3
26	12	57	-9	58.2	369	6	49	108	121	11.4	6.1
27	14	65	+16	72.2	231	4	76	74	109	16.1	2.6
28	10	42	-16	99.0	313	3	45	138	129	10.6	7.4
<i>Erythroidinized; Hyperventilation with Air</i>											
29	41	29	-25			14	11.8	62	127	7.8	9.5
30	61	38	-9	28.1	130	18	13.7	51	119	7.0	9.0
31 ³	59	12	-48	42.6	200	17	35.6	80	120	9.1	10.9
32	70	39	-14	22.2	215	40	9.8	94			
33 ³	44	13	-38	55.0	280	16	37.8	75			
<i>Erythroidinized; Hyperventilation with Oxygen</i>											
34	87	18	-26	19.2	140		17.0	54	122	8.3	9.3
35 ³	150+	16	-38	31.5	210	15	66	78			
36	150+	18		31.2	107		23.6	37			

¹ Increase or decrease from control level breathing air.² Determination of adenosine triphosphate (acid-labile and ribose monophosphate) reveals normal values in all classes of experiments except under the circumstance of low oxygen with acapnia; about 25% lower than normal values were obtained under this circumstance.³ Blood pressure decreased to a low level after blood specimens obtained.

Extreme degrees of hyperventilation in these morphinized animals induced only slight changes in the electrocorticograms.

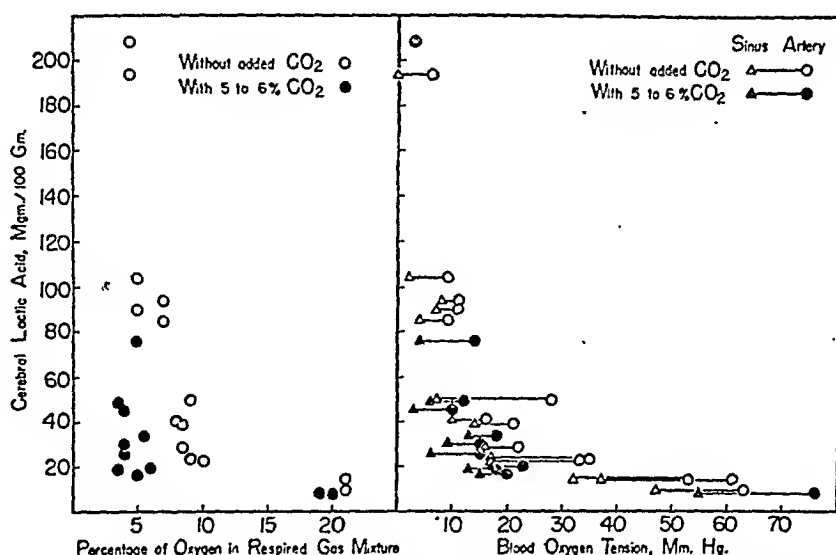


Fig. 2. RELATION OF CEREBRAL LACTIC ACID to percentage of oxygen respired and to oxygen tensions of arterial and cerebral venous blood. Effects of adding 5 to 6% carbon dioxide to the respired gas mixture.

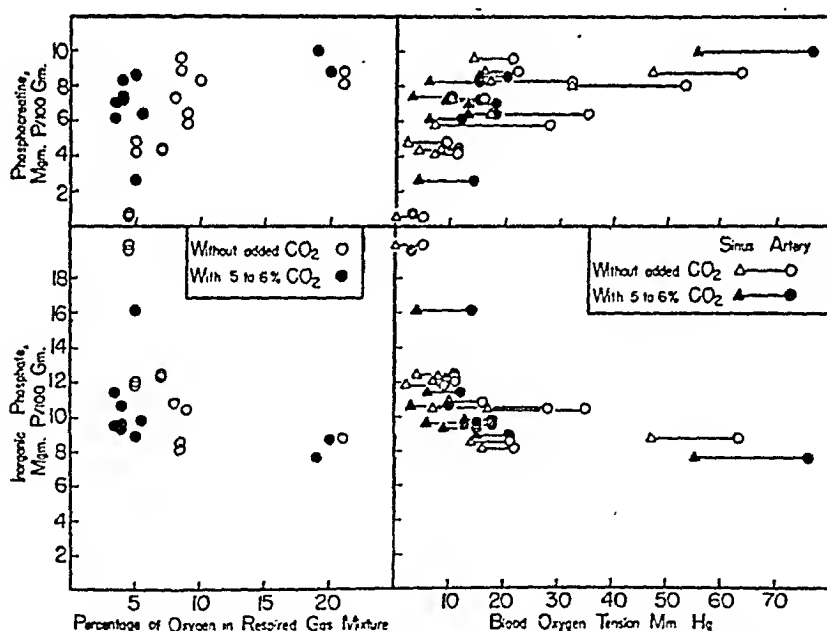


Fig. 3. RELATION OF CEREBRAL PHOSPHOCREATINE and inorganic phosphate to percentage of oxygen respired and to oxygen tensions of arterial and cerebral venous blood. Effects of adding 5 to 6% carbon dioxide to the respired gas mixture.

Hypoxia. As the oxygen in the respired air was decreased below a critical level, a progressive increase in the cerebral lactic acid occurred (fig. 2). In extreme hypoxia the cerebral phosphocreatine was partially decomposed with the formation of inorganic phosphate (fig. 3). These changes were definitely diminished by the

addition of 5 to 6 per cent carbon dioxide to the gas mixture, which improved the oxygenation of the arterial blood (fig. 4). This effect is not to be ascribed to the pH shift in the oxygen dissociation curve, since arterial oxygen tension has been plotted rather than oxygen saturation. The effect of carbon dioxide on the cerebral constituents was due in large part (but not entirely) to the improved oxygenation, as becomes evident when the levels of the cerebral constituents are plotted against the oxygen tension (figs. 2 and 3). The addition of carbon dioxide evidently permitted greater respiratory exchange with less loss of carbon dioxide. (In some experiments 5-6 per cent carbon dioxide was not sufficient to completely prevent loss of carbon dioxide, while in others a slight increase in carbon dioxide tension occurred, as shown in table 1).

When the relationship between the cerebral lactic acid and the oxygen tension of the arterial or venous blood is considered, it appears that apart from its effect on blood oxygenation the administration of carbon dioxide slightly decreased the extent of the lactic acid rise (fig. 2). A similar but less clearly defined effect on the

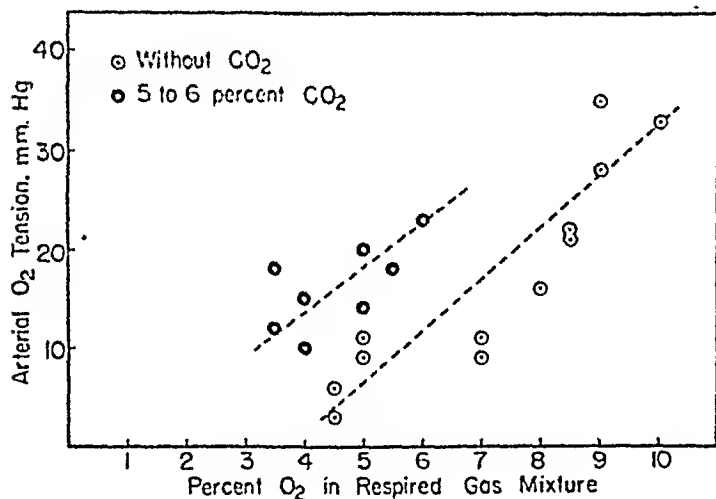


Fig. 4. EFFECT OF 5 TO 6% CARBON DIOXIDE in the respired gas mixture on the arterial oxygen tension in hypoxia.

phosphocreatine breakdown is discernible (fig. 3). Improved cerebral circulation may have been a factor, since the effects of carbon dioxide addition were more definite on the arterial side than on the venous side. However, the effects did not correlate well with the changes in carbon dioxide tension. No adequate explanation of the effects of carbon dioxide as observed on the venous side can be offered at this time.

At the lowest blood oxygen levels, however, the addition of carbon dioxide appeared to be a disadvantage. Values of arterial oxygen tension as low as 3 and 6 mm. Hg were attained in animals not given carbon dioxide, while with addition of carbon dioxide the lowest level of arterial oxygen reached was 10 mm. Hg. Three attempts to reach lower levels under these conditions resulted in death of the animals. In one of these an arterial blood specimen obtained just before death had an oxygen tension of 13 mm. Hg and a pH of 6.76.

In previous studies on hypoxia no breakdown of cerebral adenosine triphosphate was observed (1). In the present series two instances of partial decomposition of this constituent occurred (table 1). These two animals were subjected to the extreme limit of oxygen deficiency compatible with life, and probably would have ex-

pired within a few minutes had the experiment not been terminated by the freezing of the brain. The decomposition of the cerebral phosphocreatine was almost complete, and a part of the adenosine triphosphate had suffered the loss of the first acid-labile phosphate group.

The cerebral glucose and glycogen showed no significant changes during hypoxia, except that in one instance the glycogen was below the normal range.

Unfortunately the electroencephalograph was not available when the hypoxic animals of this series were under investigation. However, in a later experiment it was found that the administration of mixtures containing $3\frac{1}{2}$ to 5 per cent oxygen and 5 per cent carbon dioxide produced high-voltage slow waves, a type of record indistinguishable from that produced in the same animal by mixtures containing 6 to $7\frac{1}{2}$ per cent oxygen without carbon dioxide.

COMMENT

In a study of the metabolism of neural tissues of the cat, Craig and Beecher (20) measured the lactic acid production of slices of cerebral cortex. It was found that the rate of glycolysis increased as the oxygen tension was decreased below 140 mm. Hg. The general shape of the curve was the same as that reported here for the dog brain *in vivo*, but the accumulation of lactate does not begin *in vivo* until the oxygen tension has been reduced to less than 25 mm. Hg in the cerebral venous blood and presumably is lower still in the cerebral tissue. Elliott and Henry (21) pointed out that with tissue slices the rate of diffusion of oxygen is a limiting factor. Using homogenized brain suspensions they found that the cortical cells respire at their normal rate, with suppression of glycolysis, at oxygen tensions as low as 4 mm. Hg. Glycolysis begins at lower oxygen tensions. These results appear to be in accord with the findings *in vivo*.

Craig (22) also observed that changes in the carbon dioxide tension influence the rate of glycolysis of cortex slices. The rate was found to be higher at a carbon dioxide tension of 35 mm. Hg than at 7 mm. Hg at a constant *pH*. A further increase of carbon dioxide tension to 140 mm. Hg had no effect. On the basis of these experiments it was suggested that hypercapnia might induce an increase of the cerebral lactic acid *in vivo*. However, the present investigation has shown that no such increase occurs, nor is any decrease induced by acapnia. The physiological significance of the *in vitro* experiments is therefore open to question in this instance.

Gibbs, *et al.* (2) have studied the action of carbon dioxide in counteracting the effects of hypoxia in human subjects. They found that when carbon dioxide loss is prevented the tolerance to low oxygen is greatly increased. It was shown that carbon dioxide increased the arterial and cerebral venous oxygen tensions at comparable levels of respired oxygen. These workers believed acapnia to be the cause of the high-voltage slow waves in the electroencephalogram and the associated clouding of consciousness which occur when 6 to 8 per cent oxygen in nitrogen is respired. Their data are not quite convincing on this point, however, since when the arterial and venous oxygen tensions are calculated it appears that the levels of oxygen tension attained were never quite as low in the presence of added carbon dioxide as they were in comparable experiments without carbon dioxide.

In morphinized dogs hyperventilation with adequate oxygen did not cause appreciable slowing of the electrocorticogram such as occurs during hypoxia (1). Gas mixtures low in oxygen with added carbon dioxide produced high-voltage slow waves. Addition of carbon dioxide to the gas mixtures had only a barely perceptible influence on the changes in cerebral lactic acid and phosphocreatine at comparable levels of arterial or venous oxygen tension. It is concluded that in the morphinized dog the carbon dioxide tension is a factor of less significance than the oxygen tension in relation to cerebral function during hypoxia.

SUMMARY

Cerebral tissue from morphinized dogs was obtained for chemical analysis by freezing *in situ* with liquid air. The specimens were assayed for lactic acid, glucose, glycogen and acid-soluble phosphorus compounds. Blood samples obtained immediately prior to the freezing were analyzed for lactic acid, glucose, oxygen, carbon dioxide and pH, and the tensions of oxygen and of carbon dioxide were calculated. The effects of variations in the respired gas mixtures were investigated.

Variations of arterial carbon dioxide tension in the presence of adequate oxygen caused no significant changes in the cerebral constituents studied.

Hypoxia induced an increase of lactic acid and a breakdown of phosphocreatine in the brain. These changes were diminished when the associated acapnia was counteracted by adding carbon dioxide to the respired gas mixture. The effect of carbon dioxide was due in large part to improved oxygenation of the arterial blood. Improved cerebral circulation and unknown factors may also have contributed.

The cerebral adenosine triphosphate was found to remain undecomposed during hypoxia until the extreme limit of tolerance was reached. At this point the phosphocreatine was almost completely decomposed and decomposition of adenosine triphosphate began. The cerebral glucose remained in the normal range during hypoxia. The glycogen values also were normal with the exception of one low result occurring in extreme hypoxia.

In the morphinized dog, changes of carbon dioxide tension did not play a major rôle in the regulation of the electrocorticogram either in the presence or in the absence of adequate oxygen.

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EFFECT OF ANOXIA AND BLOOD TRANSFUSIONS ON THE PRODUCTION OF POLYCYTHEMIA IN RABBITS¹

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IT WAS the purpose of this study to determine the combined effects of repeated blood transfusions in conjunction with intermittent exposures to anoxia on rabbits as a possible method for producing a high degree of polycythemia in a comparatively short period of time. Varying degrees of polycythemia have been produced experimentally in laboratory animals by means of anoxia (1, 2) and by the administration of cobalt (3, 4). The possibility of artificially inducing a polycythemic condition in animals and man by the injection of red blood cell suspensions has been reported recently as a method for increasing their altitude tolerance (5, 6)

Boycott and Douglas (7), in 1909, while investigating the effect of whole blood transfusions on rabbits, found that with repeated transfusions in the same rabbit, each transfusion being given after an interval of several days, the added hemoglobin appeared to be destroyed at a progressively faster rate. Robertson (8) found that daily injections of 10 cc. of whole blood in rabbits depressed the activity of the bone marrow, as indicated by reticulocyte counts, and that an anemic condition increased the bone marrow activity. Campbell (9), working with rabbits, showed that he could definitely increase the tissue oxygen tension, as well as increase the hemoglobin and red cell counts by large injections of whole blood. Wetzig and D'Amour (5) produced a polycythemic condition in rats by the injection of a suspension of rat red cells in saline. More recently, Pace *et al.* (6) have reported that injections of a 50 per cent suspension of erythrocytes in a glucose and saline solution developed a polycythemia in human subjects.

METHODS

In the present experiment, 7 rabbits were studied. Four of the rabbits were given repeated whole blood transfusions along with daily exposures to anoxia, and 3 rabbits were exposed only to the anoxia. A group of donor rabbits was maintained as a source of blood for transfusions.

Since the blood transfusions were administered at frequent intervals during the course of the study, it was found convenient first to prepare both the donor and recipient rabbits by surgery so that the lateral veins on their abdomens would become greatly enlarged (10). This was done by tying off the inferior vena cava between the liver and the right renal vein to a caliber of 1.8 mm. It was a simple procedure then to withdraw blood and to inject blood into these enlarged vessels on the surface of the abdomen. About two weeks were required for the rabbits to recover fully from the operation and for the abdominal veins to develop to a suitable size.

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Blood transfusions were administered daily, when possible, to 3 of the rabbits during the course of the experiment. Powdered heparin was used as an anti-coagulant. One other rabbit received a short series of 10 transfusions during the first two weeks of the experiment, and then was subjected only to the anoxia for the rest of the study.

The hematocrit values were measured in Wintrobe tubes which were centrifuged at over 2000 r.p.m. for 30 minutes. Hemoglobin determinations were made by the acid hematin method with a Klett-Summerson colorimeter. The body weights of the rabbits were determined each day and used as an indication of their state of health.

The anoxia was induced by subjecting the rabbits to low atmospheric pressures in a decompression chamber. The rabbits were exposed to the anoxia for 16 hours each day, 5 days a week. The simulated altitudes ranged from 18,000 to 32,000 feet, depending on the health of the rabbits and the degree of polycythemia that developed. The rabbits remained at ground level over the week-ends which permitted a certain amount of recuperation.

RESULTS

When blood transfusions were administered to 4 rabbits in conjunction with exposures to low atmospheric pressures, the hematocrits increased from an average normal value of 39.1 per cent to an average maximum value of 84.1 per cent in an average of 48.8 days. The maxima ranged between 80 per cent and 90.2 per cent. The hematocrits of 3 rabbits that were subjected to only the anoxia, and which received no blood transfusions, increased from an average normal value of 41.1 per cent to a maximum of 72.0 per cent in 68.3 days. The maximum hematocrit values ranged between 67.2 per cent and 78.3 per cent.

Typical results of the experiment are shown in figures 1, 2, and 3, which are the graphic records for two rabbits that received both blood transfusions and anoxia and one rabbit that was subjected only to the anoxia. Figure 1 is the record of a rabbit that was intermittently exposed to reduced atmospheric pressures for a period of 135 days (approximately 1296 hours at altitudes between 18,000 and 32,000 feet). The effect of the blood transfusions on this rabbit was shown by the immediate increase in both the hematocrit and hemoglobin values when the transfusions were administered and moderate decline of them at the end of the series of transfusions.

Figure 2 shows the results that were obtained by intermittently exposing a rabbit to anoxia for a period of 175 days (approximately 1280 hours at altitudes of 18,000 to 32,000 feet). Since the blood transfusions were continued at frequent intervals throughout the study, the hematocrit and hemoglobin values remained at high levels. On the 88th day, when the hematocrit value for this rabbit was 90.2 per cent, the exposures to anoxia and the transfusions were stopped for a while, because of a marked loss in body weight. During this period of recuperation the hematocrit decreased to 70 per cent and then increased to over 80 per cent when the anoxic stimulus and transfusions were resumed. The health of the rabbit did not improve adequately, and it died on the 175th day of the study.

The effect of anoxia alone in producing polycythemia in a rabbit is shown by

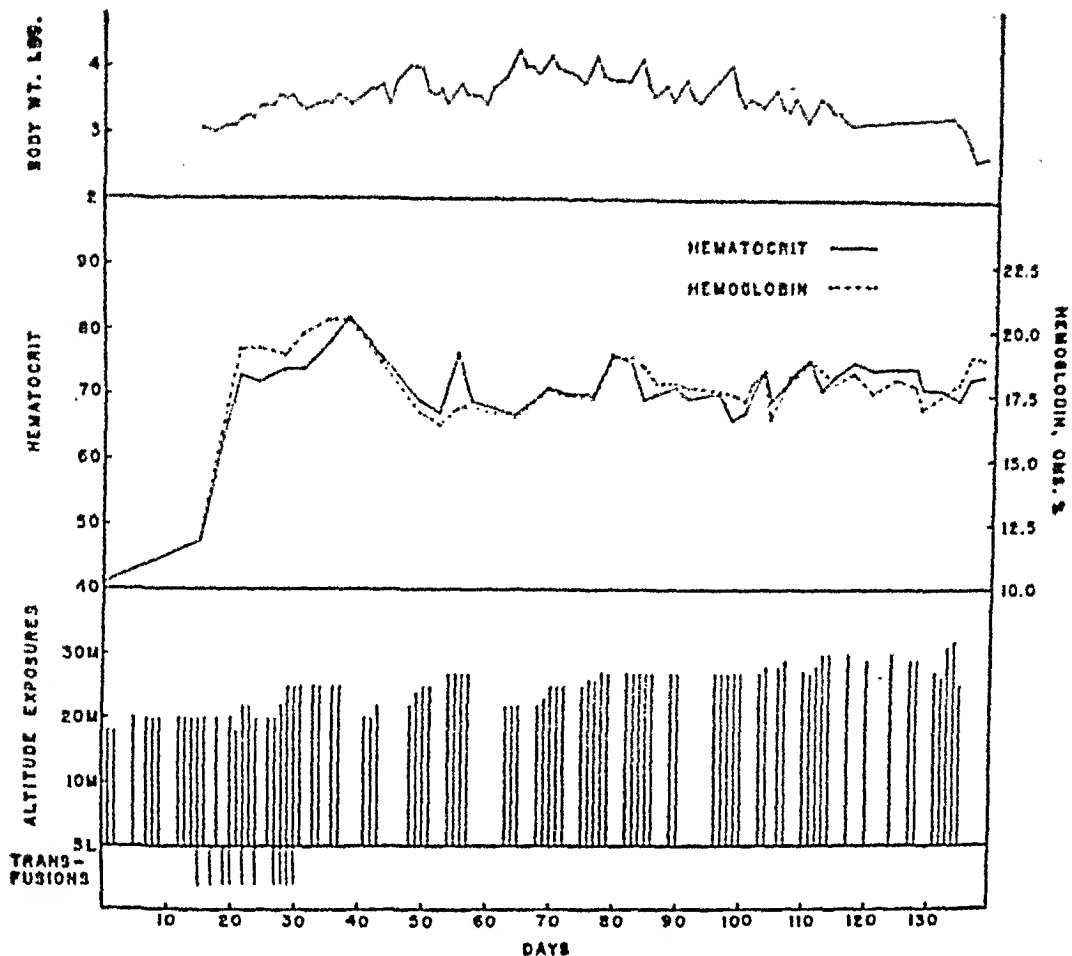


Fig. 1. EFFECT OF ANOXIA and 10 blood transfusions on the hematocrit, hemoglobin and body weight of a rabbit.

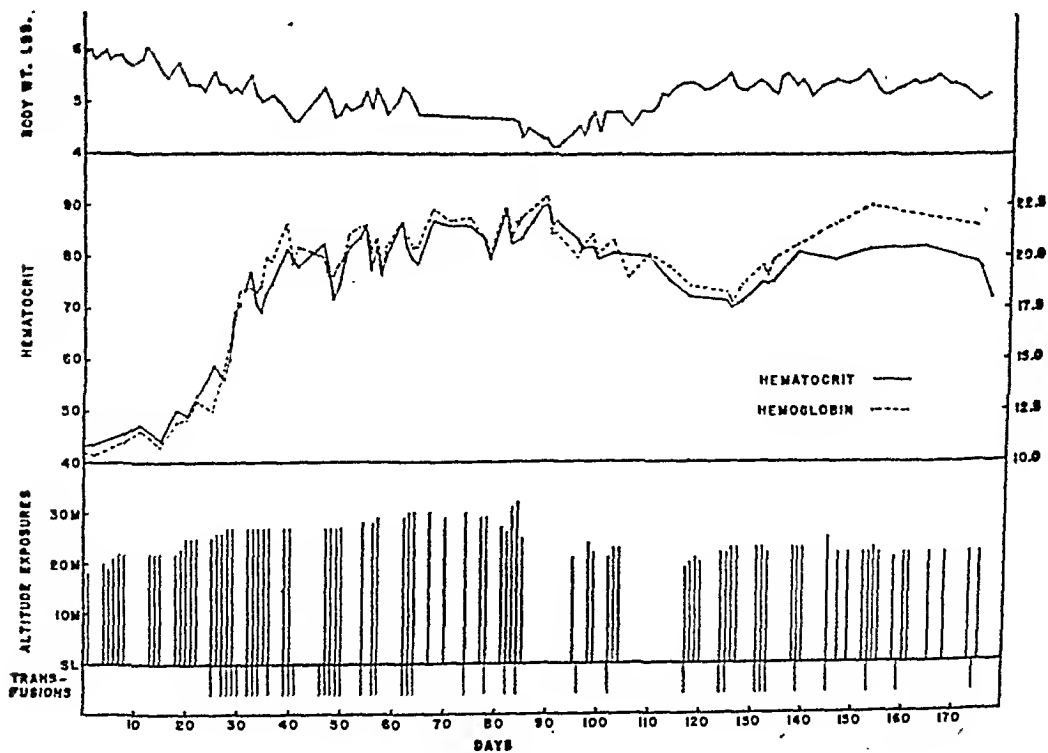


Fig. 2. EFFECT OF ANOXIA and 40 blood transfusions on the hematocrit, hemoglobin and body weight of a rabbit.

figure 3. This rabbit was exposed intermittently to reduced atmospheric pressures for a period of 99 days (approximately 613 hours at altitudes of from 19,000 to 25,000 feet). The hematocrit and hemoglobin values never reached the high levels that were obtained with the combined actions of anoxia and blood transfusions.

In general, the hematocrit and hemoglobin values tended to increase and decline in equal proportions as a result of both the anoxia and blood transfusions, which agrees with the conclusions of Van Liere (11) regarding the relationship between the hemoglobin and erythrocytes.

The growth of the rabbits, as shown by their body weight determinations, was inhibited in varying degrees by the combination of anoxia and blood transfusions,

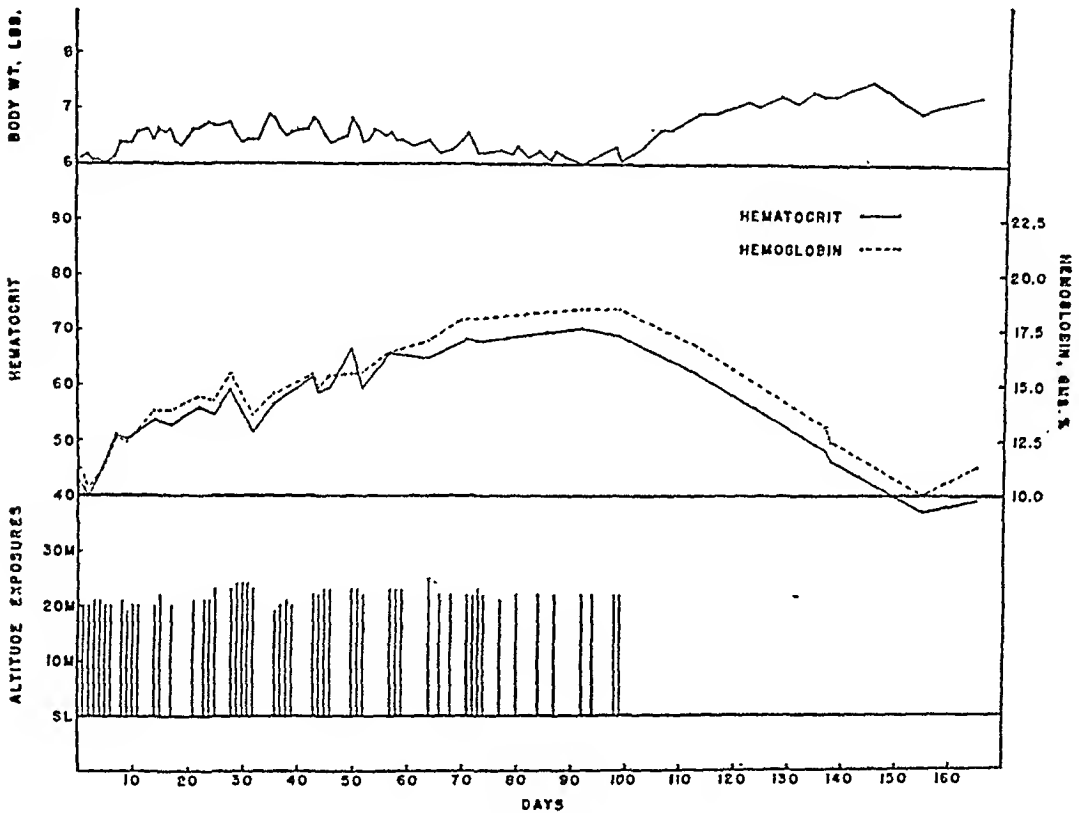


Fig. 3. EFFECT OF ANOXIA only on the hematocrit, hemoglobin and body weight of a rabbit.

and by the anoxia alone. The rabbits either failed to gain weight or actually lost weight when they were subjected to the anoxia and transfusions, and if these were stopped the body weights increased.

The general health of the 4 rabbits that were given the blood transfusions appeared to deteriorate progressively during the course of the experiment, and in each case the study of these rabbits was terminated by their deaths. They died in 26, 49, 136, and 177 days, respectively. At these times their hematocrits were all above 80 per cent. Their body weights were well below the original levels and they all had an emaciated, apathetic appearance. It is interesting to note that the blood urea that was determined for one of these rabbits at this time was over 400 mg. per cent.

On the other hand, all of the 3 rabbits that were exposed only to the anoxia survived the experiment. Although their growth was inhibited, they did not appear to be in a particularly unhealthy condition. Their hematocrit values were not as high as the transfused rabbits, but nevertheless their hematocrits had increased an average of approximately 75 per cent above their original levels.

The ability of the rabbits to withstand excessively high altitudes was shown by 2 rabbits that were repeatedly subjected to altitudes above 27,000 feet during their daily 16-hour exposures (figs. 1 and 2). These two rabbits survived six 16-hour exposures above 30,000 feet, one of which was at 32,000 feet. Thorn *et al.* (2) in 1942 showed that normal rabbits, when subjected to a simulated altitude of 25,000 feet for four hours, had a mortality rate of approximately 75 per cent with most of the fatalities occurring during the first or second exposure.

The results indicate that the combination of anoxia and blood transfusions increased the number of red cells and the amount of hemoglobin to a greater extent and in a shorter period of time than just the anoxia alone. This suggests that by limiting the transfusions to a number just sufficient to produce this immediate increase and by maintaining the polycythemia with only the anoxia, the health of the rabbits could be preserved much better than with the continued transfusions.

SUMMARY

Blood transfusions combined with exposures to anoxia produced a higher degree of polycythemia than the anoxia alone. However, the administration of blood transfusions was more detrimental to the health of the rabbits than was the anoxia alone, and continued transfusions resulted in the death of the rabbits. Altitude tolerance of the rabbits increased as the hemoglobin and hematocrit values increased, regardless of whether the polycythemia was produced by anoxia alone or by blood transfusions and anoxia.

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EFFECT OF EXTRACELLULAR ELECTROLYTE DEPLETION ON BRAIN ELECTROLYTE PATTERN AND ELECTRO-SHOCK SEIZURE THRESHOLD¹

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DARROW and Yannet (1-3) have shown in cats that extracellular electrolyte depletion produced by placing isosmolar glucose solution in the peritoneal cavity, in amounts equal to 10 per cent of the body weight, is accompanied by a decrease in the extracellular fluid volume and an increase in the intracellular fluid volume of liver and muscle tissue. In brain tissue they found no change in total water or distribution of water, and concluded that brain cells adjust to extracellular electrolyte depletion by releasing intracellular potassium. In all of the observations of Darrow and Yannet the tissues were removed for analysis 24 hours after the experimental procedure.

In the course of employing the technic of Darrow and Yannet we observed that a marked reduction occurs in electroshock and chemoshock convulsive thresholds in the first 4 hours and that thresholds returned well toward normal in 24 hours (4, 5). Therefore we investigated the changes in water and electrolyte distribution in brain occurring within the first 4 hours in order to observe early changes which might be associated with altered brain function.

METHODS

Adult male albino rats obtained from the Carworth farm and maintained on a stock diet were used. Food and water were withheld during the experimental period. The electroshock threshold was determined with an Offner 60-cycle alternating current apparatus utilizing Spiegel corneal electrodes (6). The details of this procedure have been published in full elsewhere (4).

To produce changes in seizure threshold and in electrolyte and water distribution, 5.5 per cent glucose solution was injected intraperitoneally in a dose of 10 cc/100 gm. body weight. The fluid was withdrawn at the end of 2 or 4 hours. In some experiments electrolyte was subsequently replaced by injecting intravenously the requisite quantity of sodium chloride in a 20-per cent solution.

Animals were decapitated for tissue analysis 10 minutes after intravenous in-

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jection of 0.2 cc. heparin solution. The cerebral tissue was separated from the remaining brain by extending the longitudinal fissure into the lateral ventricles. The isolated hemispheres were blotted free from blood and spinal fluid and placed in a tared weighing bottle. Hemispheres from 2 or 3 rats were pooled. Blood of the same animals was likewise pooled.

Plasma specific gravity was determined by the falling drop method of Barbour and Hamilton (10). Weighed samples of plasma and cerebral cortex were dried to constant weight at 105°C. and the total water calculated by difference. Chloride was determined by the method of Van Slyke and Sendroy (8). In the case of brain chloride preliminary alkaline digestion described by Sunderman and Williams (9) was used. For determination of sodium and potassium, 0.1 gm. of powdered dry brain tissue or 1.0 cc. of plasma was transferred to a platinum crucible, moistened with 4 N H_2SO_4 and dry ashed at 500°C. for 8 hours. The residue was dissolved in concentrated HCl, evaporated to dryness at 80°C., dissolved in the appropriate volume of water and analyzed by means of a Perkin-Elmer flame photometer (model 18). Reproducible results were obtained with this instrument when the gas and air pressures were properly adjusted and the instrument frequently calibrated with standards having electrolyte concentrations in the range of the samples being analyzed. The details of these adjustments have been described by Hald (7). Although these precautions were observed, it was difficult to determine plasma potassium accurately because of the high concentration of sodium present.

The concentrations of water and electrolyte in the extracellular (chloride space) and intracellular (chloride-free space) fluid compartments of cerebral cortical tissue were calculated by the method described by Hastings (12, 13).

RESULTS

The observed changes in electroshock threshold, specific gravity, and water and electrolyte distribution in plasma and cerebral cortical tissue which followed electrolyte depletion, replacement and spontaneous recovery are shown in table 1. The electroshock thresholds were determined in 53 rats. Water and electrolyte data were obtained from the analyses of pools of the brain and plasma of 30 groups of rats, distributed as shown in the table.

It may be seen that four hours after the intraperitoneal injection of isosmolar glucose the threshold for minimal electroshock seizures is lowered from 27 to 12 m.a. If at this time the calculated amount of electrolyte withdrawn by paracentesis is replaced by an intravenous injection of 20-per cent sodium chloride solution, the threshold rises to 15 per cent above normal within 30 minutes. If no replacement is attempted the threshold slowly rises and is almost normal after 24 hours.

The decrease in plasma water is maximal by the end of 4 hours. This decrease is accompanied by an increase in specific gravity of the plasma. According to Moore and Van Slyke (11) the specific gravity of plasma is a linear function of its protein content. Therefore, the decrease in plasma water must be accompanied by an increase in plasma protein. The consequent decrease in the Donnan factor results in a greater loss of chloride (19.2%) than sodium (17.3%) from the plasma. Considerable but incomplete spontaneous recovery occurs by the 24th hour. However,

TABLE 1. OBSERVED ELECTROSHOCK THRESHOLD, SPECIFIC GRAVITY, WATER AND ELECTROLYTE DISTRIBUTION OF PLASMA AND CEREBRAL CORTICAL TISSUE

PROCEDURE	ELECTROSHOCK ¹ THRESHOLD		PLASMA PER KG.						CEREBRAL CORTEX PER KG. MOIST TISSUE			
	No. rats	m.a.	No. samples pooled	Specific Gravity	H ₂ O gm.	Cl mEq.	Na mEq.	K mEq.	H ₂ O gm.	Cl mEq.	Na mEq.	K mEq.
Control	17	27 ± 0.8	8	1.0245	929 ± 2.32	104 ± 0.92	139 ± 0.71	5.3 ± 0.24	790 ± 0.78	34.6 ± 0.38	46.3 ± 0.34	98.4 ± 0.98
2 hr. after i.p. glucose	5	15.6 ± 0.9	8		914 ± 0.34	88.3 ± 1.16	116 ± 1.31	4.9 ± 0.35	792 ± 0.87	28.9 ± 0.47	40.0 ± 0.61	89.3 ± 1.32
4 hr. after i.p. glucose	17	12 ± 0.4	8	1.0314	909 ± 0.26	84.1 ± 1.28	115 ± 3.16	6.5 ± 0.45	792 ± 3.71	27.0 ± 0.57	39.2 ± 0.48	88.9 ± 1.29
24 hr. after removing i.p. glucose	12	23 ± 1.5	3	1.0295	917 ± 0.58	98.6 ± 2.13	132 ± 1.52	7.1 ± 0.66	788 ± 0.91	32.9 ± 0.38	46.1 ± 1.04	86.6 ± 1.10
30 min. after hypertonic NaCl, 4 hr. after i.p. glucose	3	31.8 ± 4.2	3		931 ± 1.48	106 ± 1.22	139 ± 1.22	3.92 ± 0.13	784 ± 0.94	37.5 ± 0.09	43.9 ± 0.66	95.9 ± 1.09

¹ 0.2 sec. stimulus duration; 60-cycle A.C.; Spiegel corneal electrodes. ± Standard error.

plasma sodium and chloride concentrations are normal 30 minutes after the intravenous replacement of lost electrolyte with the calculated amount of sodium chloride, but the potassium concentration is reduced to less than normal.

In brain, the loss of extracellular electrolyte produces a decrease in the quantity of sodium, chloride and potassium. The deficit in sodium and chloride is largely restored after a 24-hour recovery period or within 30 minutes following replacement of lost electrolyte. The brain potassium is further reduced during the period of spontaneous recovery, and is only partially restored 30 minutes after the replacement of electrolyte with the requisite quantity of sodium chloride. There is no significant change in the total brain water content during electrolyte depletion or spontaneous recovery. However, 30 minutes following the molar replacement of depleted extracellular electrolyte with sodium chloride, a slight but significant reduction in total brain water occurs.

In order to determine the distribution of water and electrolyte in the intra- and extracellular spaces, we have used the calculations described by Hastings (12, 13). These calculations are based on the assumption that chloride space may be estimated from the concentration of chloride in plasma. It is well known that chloride space is not identical with the extracellular space, but several investigators (13-15) have concluded that volume changes in the two spaces are parallel. Therefore, chloride space offers a reliable index for estimating directional changes in the volume of extracellular fluid. The results of these calculations are shown in table 2.

Most of the observed alterations in water and electrolyte distribution are not closely correlated with the changes in convulsive threshold. The data show that a small but not significant increase in total brain water occurs concomitantly with a decrease in electroshock seizure threshold and, conversely, a decrease in total brain water is associated with a return of electroshock threshold to normal and above. However, the parallelism between the change in volume of total brain water and the electroshock seizure threshold is not immutable. Within 24 hours after extracellular electrolyte depletion the brain loses more water than it had initially gained, but at this time the electroshock seizure threshold is still approximately 20 per cent below normal.

The electroshock seizure threshold is not closely correlated with the intracellular fluid volume and is independent of the extracellular fluid volume. As shown in table 2, extracellular electrolyte depletion sufficient to reduce seizure threshold by 56 per cent significantly increases brain cell volume. However, 24 hours after extracellular electrolyte depletion the intracellular fluid volume is back to normal, but the electroshock seizure threshold is only 80 per cent of normal. Extracellular fluid volume is reduced as a result of extracellular electrolyte reduction and returns to normal after a 24-hour recovery period. Extracellular fluid volume is increased following electrolyte replacement. However, we have previously shown (4) that the extracellular fluid volume may be greatly increased by the oral administration of isotonic sodium chloride solution without significant change in seizure threshold.

Loss of extracellular electrolyte is accompanied by a significant decrease in brain intracellular potassium. The potassium concentration continues to fall during the 24 hours following extracellular electrolyte loss, although seizure threshold returns

TABLE 2. DERIVED WATER AND ELECTROLYTE DISTRIBUTION IN EXTRACELLULAR WATER AND CEREBRAL CORTICAL TISSUE

PROCEDURE	CONCENTRATION OF ELECTROLYTE PER KG. EXTRACEL. WATER			CORTICAL FLUID PER KG. OF TISSUE		CATION IN CHLORIDE-FREE SPACE PER KILO OF CORTICAL TISSUE	
	Cl mEq.	Na mEq.	K mEq.	Chloride space gm.	Chloride free space gm.	Na mEq.	K mEq.
Control	117 \pm 1.05	142 \pm 0.70	5.4 \pm 0.24	294 \pm 2.18	496 \pm 1.89	4.4 \pm 0.36	96.7 \pm 1.00
2 hr. after i.p. glucose	101 \pm 1.22	121 \pm 1.36	5.1 \pm 0.37	288 \pm 7.28	504 \pm 7.49	5.3 \pm 0.86	87.8 \pm 1.29
4 hr. after i.p. glucose	97.9 \pm 1.48	119 \pm 3.35	6.8 \pm 0.47	276 \pm 5.26	516 \pm 5.37	6.4 \pm 1.09	87.0 \pm 1.36
24 hr. after removing i.p. glucose	112 \pm 2.38	137 \pm 1.64	7.3 \pm 0.06	294 \pm 7.13	493 \pm 6.75	5.8 \pm 0.59	84.4 \pm 1.08
30 min. after hypertonic NaCl, 4 hr. after i.p. glucose	118 \pm 1.52	142 \pm 1.20	4.0 \pm 0.14	317 \pm 3.19	466 \pm 2.35	6.5 \pm 0.60	94.7 \pm 1.07

toward normal. No evidence is found in these data to indicate any clear relation between intracellular cation concentration and electroshock seizure threshold.

Of all the factors studied, extracellular sodium and chloride concentrations showed the best correlation with electroshock threshold, as may be seen in tables 1 and 2. Further experiments will be needed to isolate the separate rôle of these two ions.

DISCUSSION

Unfortunately one cannot be certain that present methods of analysis give even a relative estimate of intracellular fluid volume. The extracellular space of brain, when determined *rapidly* (8 minutes) with radio-active sodium, is less than 5 per cent of the total brain water (16). If adequate time is allowed for the radio-active sodium fully to equilibrate (17 hours), the extracellular space is found to be 30 per cent (17). This latter figure is in agreement with the extracellular volume estimated from the sodium or chloride concentration. However, Toman and Goodman (18) have suggested that estimation of extracellular space from sodium or chloride concentration might be invalidated by the possibility that some cells (glia?) contain sodium and chloride. The existence of an extracellular electrolyte pattern within such cells would compromise the indirect methods used to measure the volume of those remaining cells (neurones?) which are postulated to be relatively free of both sodium and chloride.

It can be seen from table 2 that potassium leaves the calculated intracellular space when external sodium is low, and returns when the sodium concentration is restored to normal. This behavior with respect to potassium is typical of muscle, kidney and other body cells, but these cells apparently differ from brain in that they shrink or swell in accordance with changes in the extracellular sodium concentration (19). Perhaps entirely new technics or approaches will be required to determine whether cerebral neurones undergo corresponding volume changes in response to alterations in extracellular electrolyte concentration.

SUMMARY

Data are presented showing the distribution of water, sodium, potassium and chloride in the plasma and brains of rats subjected to acute extracellular electrolyte depletion and subsequent replacement, and the relation of these alterations to concomitant changes in electroshock seizure threshold. Although it was not possible from these data to identify definitively those aspects of water and electrolyte balance which determine the reduction in electroshock seizure threshold, the following tentative conclusions are drawn: 1) The electroshock seizure threshold is not closely correlated with total brain water or the calculated intracellular fluid volume. 2) The electroshock seizure threshold is independent of the volume of extracellular fluid. 3) The electroshock seizure threshold is independent of the intracellular potassium concentration. 4) The electroshock seizure threshold is more closely correlated with extracellular sodium and chloride concentration than with other factors examined.

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OXYGEN CONSUMPTION CORRELATED WITH THE THERMAL REACTIONS OF YOUNG RATS TO ERGOTOXINE¹

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THERMAL responses to the alkaloids of ergot have been studied by numerous investigators, and the results reported have shown considerable qualitative and quantitative variation. Rather extensive reviews of the literature have been made by Barger (1) and White (2). Ergotoxine has quite consistently produced hyperthermias in the rabbit (2-6). Githens (5) observed hypothermic reactions in 7 mice, and White (2), in some 600 mice, always observed a fall in temperature when the dose was at all effective. Whereas ergotamine has been shown by Rothlin (3) to produce hypothermic reactions in small doses and hyperthermic reactions in large doses in the same species (rabbits), ergotoxine always produces hyperthermia with an effective dose. The present report will be confined to ergotoxine, since the authors feel that attempts to extrapolate the results in terms of ergotamine should be made with great caution.

The reactions of albino rats to ergotoxine have been investigated in connection with other studies of temperature regulation in this species and initial hyperthermic reactions have been consistently observed in adult animals of Wistar and Denver University strains following intraperitoneal administration of 4.5 mg. of ergotoxine ethanesulfonate per kilogram of body weight when the environmental temperature was 28° to 31°C. (7). These results are not in agreement with those of Githens (5), who reported hypothermic responses in 8 rats following subcutaneous injections of 4.0 mg/kg. of ergotoxine phosphate. Some of the factors which are possibly responsible for the discrepancy between our results and those of Githens have been discussed previously (7).

The action of ergotoxine on the thermoregulatory centers in the hypothalamus has been accepted generally as being the primary mechanism responsible for the hyperthermal reaction (8). That this is a valid opinion is supported by the observations that the drug produces varying degrees of hypothermia in young rats and hyperthermias in those animals that have attained the age range within which the hypothalamus is well myelinated and thermoregulation has become efficient (9).

The effect of ergotamine on oxygen consumption has been investigated both clinically and in experimental animals, chiefly because of the former interest in the therapeutic use of the drug in hyperthyroidism. This literature has been reviewed carefully by Barger (1) and the present authors subscribe to his opinion that, since ergotamine differs from ergotoxine as regards thermal effects in certain species, the metabolic effects may also be different.

In his review of the literature, Barger found only the work of von Euler (10) to be related to the effect of ergotoxine on tissue metabolism. This investigator, using minced frog muscle, found that ergotoxine brought about the most rapid decolorization of methylene blue at a concentration of 10^{-12} . It appears from this work that ergotoxine may directly stimulate dehydrogenase activity in muscle. In a perfusion experiment using the isolated hind limb of a dog, von Euler (11) observed

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complete inhibition of the metabolic stimulating effect of adrenalin; this was indicated by the observation that the 20 per cent increase in oxygen consumption produced by perfusion with a $10^{-9.3}$ concentration of adrenalin, was superseded by a slow decline in oxygen consumption in the presence of ergotoxine. Other than those mentioned above, the authors are aware of no report on oxygen consumption as affected by ergotoxine.

The present study of oxygen consumption represents an attempt to determine the relationship between metabolic and thermal responses to ergotoxine. It seemed that oxygen consumption in animals of both pre- and post-regulatory age ranges should be investigated since the former have been shown to react to ergotoxine with varying degrees of hypothermia and the latter with hyperthermia.

MATERIALS AND METHODS

Four litters of Wistar strain and 2 litters of Sprague-Dawley strain albino rats were studied at ages ranging from 12 to 37 days. Each animal was given a single intraperitoneal injection of ergotoxine ethanesulfonate (4.5 mg/kg. from a solution prepared by dissolving 1 mg. in 2. cc. of 6.25% ethyl alcohol), after which temperature and oxygen consumption were observed for 2 hours. Individual animals from each of 3 litters were studied on the 12th, 16th, 20th, 23rd, and 26th days of age and from the other 3 litters on the 16th, 20th, 23rd, and 26th days. In addition, animals from some litters were studied on the 28th, 32nd, and 37th days of life. Control animals from one litter were studied on the 12th, 16th, 20th, 23rd, and 26th days of age.

Temperatures during the experiments were recorded at one-minute intervals from each of 2 three-unit thermopiles by means of a two-channel Brown electronic recording potentiometer. One of the thermosensitive elements was placed in the high colonic region of the animal, and the second recorded the temperature of the air chamber which consisted of a glass jar of 3-liter capacity. The latter thermopile was placed midway between the cage containing the animal and the wall of the chamber. The chamber was immersed in a water bath kept in a thermostatically regulated oven. During a given experiment the temperature change of the chamber was always less than $1^{\circ}\text{C}.$, and all experiments were conducted at an environmental temperature range of 28.5 to $30.5^{\circ}\text{C}.$

Approximately 250 grams of moist soda-lime were used in the jar to absorb the CO_2 produced. Since it was not feasible to quantitate the CO_2 produced, respiratory quotients were not determined. After the animal had been confined to the chamber a small amount of oxygen was admitted to the jar to replace that which had been initially used by the animal and to bring the oxygen concentration to approximately 20 to 22 per cent. The oxygen consumption was recorded by means of a small spirometer and long smoked belt. The marking tip of the spirometer consisted of a finely drawn glass rod (with a small glass beaded tip) suspended from a fine watch spring. In general the consumption was studied during 6-minute intervals and oxygen was readmitted to the chamber at 6-, 12-, or 18-minute intervals, depending upon the rate of consumption of the animal. Slight changes in chamber temperature and consequent gas volume changes were corrected for by reading the temperature change in the interval and utilizing an experimentally determined constant. Following an observation on normal temperature and oxygen consumption, the intraperitoneal

injection was made without removing the thermocouple from the animal, so that ensuing observations began very soon after injection and without additional trauma.

An estimate of respiration was obtained in the older animals by observing the change in the meniscus of a partially filled tube. The end of the tube was in the bottom of a small flask partially filled with water. The air line from the chamber passed into this small flask and then to the spirometer.

The average decrease in temperature in pre-regulatory rats during a 2-hour interval of hypothermia was determined by finding the area under the time-temperature curve in degree-minutes by means of a planimeter and dividing by 120 minutes. Similarly the average increases in temperature during hyperthermic reactions of regulatory animals were obtained by dividing the areas by the time the hyperthermia lasted.

Oxygen consumption was computed for each interval in liters per square meter per hour, by using the surface area formula, $A = 9.0 (W)^{2/3}$, where A is the area in sq. cm. and W the weight in gms. Average increase or decrease in oxygen consumption was computed by the same method as that described above for temperature changes. This average change was then computed in terms of percentage of normal consumption in the same animal where normal consumption was taken at 100 per cent.

RESULTS

Within litters, animals manifested hypothermias with ergotoxine up to an age ranging from 20 to 28 days, after which hyperthermias became manifest. Animals of a given litter older than the first animal displaying hyperthermia always displayed hyperthermias. As previously reported (13, 9), this corresponds with the age range in which regulation against cold in Wistar strain animals has been shown to become efficient. Typical thermal and oxygen consumption reactions of Wistar strain animals at 16 and 26 days of age are shown in figure 1, together with records of controls of the same ages.

In the 4 litters of Wistar strain animals the hypothermias following administration of ergotoxine were accompanied in each case by decreased oxygen consumption. The degree of hypothermia was, in general, directly related to the degree of decreased metabolism, as indicated by the experimental values and the least squares line shown in the third quadrant of figure 2.

The hypothermias were, in general, associated with decreased muscular activity, while hyperthermias were associated with considerable increase in muscular activity. The 12- and 16-day old animals, as a rule, reacted with greater hypothermic reactions and greater decreases in oxygen consumption than did older animals in the pre-regulatory group.

The young animals of the Sprague-Dawley strain did not show a correlation of decreased oxygen consumption accompanying the hypothermias. In fact, 4 of the young animals of this strain had slight increases in oxygen consumption during rather marked hypothermia (second quadrant of figure 2).

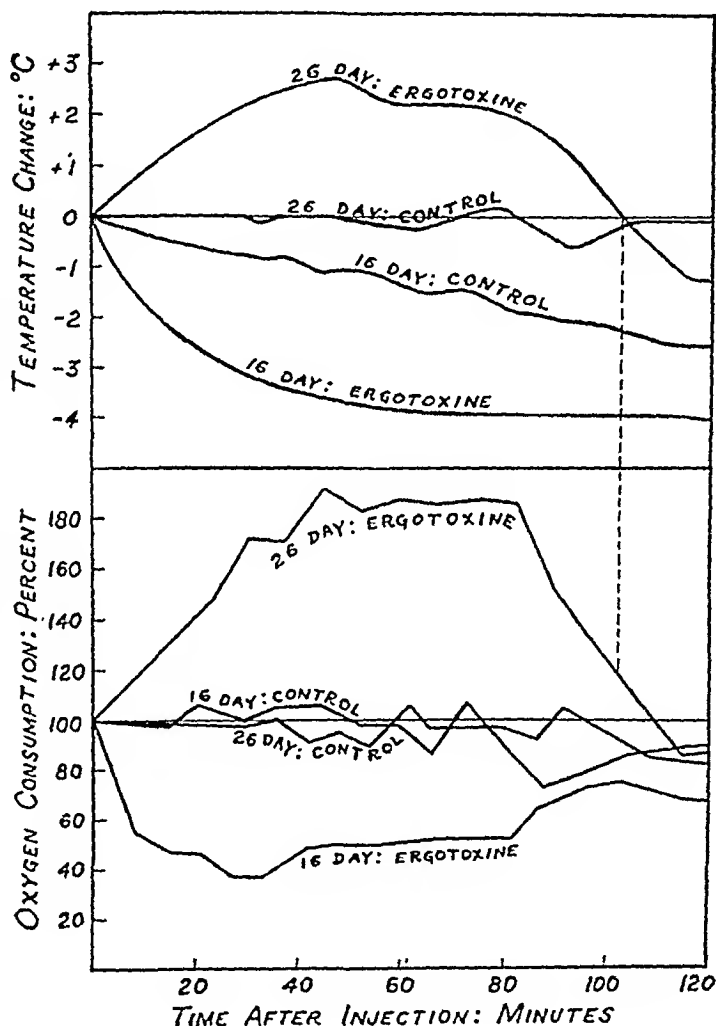
Hyperthermias were found to have a linear relation to increased oxygen consumption in both strains of rats, as indicated by the experimental values and the least squares line in the first quadrant of figure 2. One observation was not included

in the regression line; this was the point 1.17°C . and 208 per cent oxygen consumption. The hyperthermias were consistently accompanied by increased amplitude of respiration; this was considerably greater than changes in respiration observed in control animals under the same conditions.

DISCUSSION

Herrington (12) has shown that metabolism in rats is at a minimum level when their environmental temperature ranges between 28.5° and 30.5°C . The temperature range of the chamber utilized for the present investigation was maintained within

Fig. 1. TYPICAL THERMAL AND OXYGEN CONSUMPTION RESPONSES of 16- and 26-day rats given intraperitoneal injections of ergotoxine (4.5 mg/kg.), and those of litter mate controls. The vertical broken line emphasizes the fact that the temperature of the 26-day-old rat had returned to normal while the oxygen consumption was elevated approximately 20%.



this range and it has therefore been assumed that oxygen consumption in our rats was little, if at all, influenced by environmental temperature.

Hypothermic reactions of Wistar strain animals appear to be significantly related to decreased oxygen consumption as indicated by the application of an *F* test which gave a value for *P* of approximately 0.01 when the linear regression of the least squares line was compared with the variance about the regression line. The decreased oxygen consumption does not necessarily indicate that the drug interferes with metabolism for, by the independent operation of van't Hoff's Law, it could have resulted entirely from the decrease in body temperature. The relation of changes in

body temperature to metabolic rate has been studied by DuBois (14) in 182 human cases of 'heterogeneous fevers'; he arrived at an average reaction rate constant of 2.3 which represents a change in metabolic rate of 13 per cent for each degree (C.) change in body temperature. In our Wistar strain rats the percentage change in metabolic rate per degree (C.) change in temperature was 11.8 per cent; this value is obviously so near that of DuBois, referred to above, that a definite statement regarding direct interference with metabolism by ergotoxine cannot be made. Decreased activity might be considered as a possible factor in the decreased metabolism of these ergo-

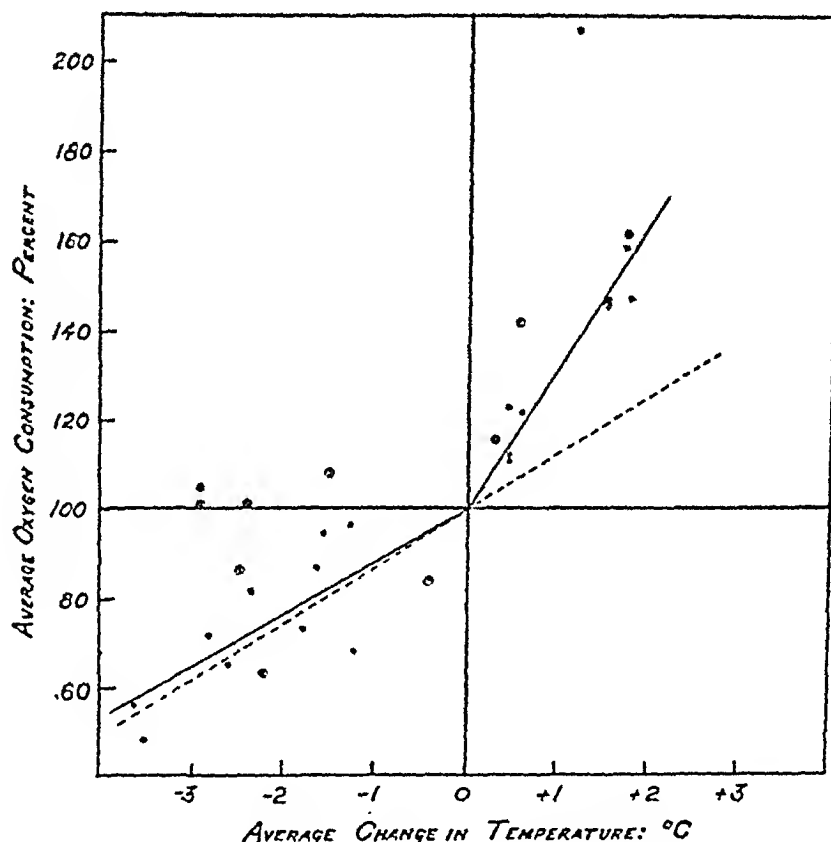


Fig. 2. THE LEAST SQUARES REGRESSION LINE in the first quadrant represents the relation of increased body temperature to increased oxygen consumption (Wistar strain: dots, Sprague-Dawley strain: circled dots). The least squares line in the third quadrant was found from values of Wistar strain animals of preregulatory age range. The preregulatory Sprague-Dawley rats showed lack of correlation of hypothermia and decrease in oxygen consumption. The broken line represents the relation between the variables that might be anticipated from the independent operation of van't Hoff's Law.

toxine-treated rats but this appears to be contraindicated by the fact that they exhibit pronounced hypersensitivity to sound and touch.

The hypothermic responses in Sprague-Dawley rats were accompanied by variable oxygen consumption values (fig. 2). The 4 points in the second quadrant of figure 2 indicate a stimulation of metabolism with concomitant increase in heat loss while the 3 points in the third quadrant illustrate essentially the same types of response displayed by Wistar strain rats.

The hyperthermias, in all cases except one, showed an essentially linear relation to oxygen consumption (fig. 2) as indicated by the least squares regression line slope of 32.5 per cent per degree (C.). The regression is highly significant, for P was found to be less than 0.01 by the test mentioned above. Since this regression line has a slope 2.5 times as great as that which would be anticipated on the basis of operation of van't Hoff's Law (with the constant mentioned above), it appears that the hyperthermias result from increased heat production through a primary stimulation of metabolism. The extent to which increased muscular activity may be a factor in the increased metabolism has not been accurately determined.

It has been assumed that the hyperthermias produced by ergotoxine are due both to increased heat production and decreased heat loss (5, 8). The conclusion of Githens (5) that the 'coldness of the skin' (in rabbits) suggests reduced heat dissipation is supported by evidence of direct vasoconstrictor action of the alkaloids on peripheral vessels (3). Panting, however, is of primary importance in heat dissipation in furred animals, as has been shown by Dill *et al.* (15) and others. Polypnea has been observed by White (2) during hyperthermias induced in rabbits by ergotoxine, and this author states that the resultant dissipation of heat may account for the variability of hyperthermic responses. In the present experiments dissipation of heat through increased respiration appears to outweigh any heat conservation that may result from vasoconstriction. This was indicated by the observations in nearly all cases that when body temperatures had returned to normal the oxygen consumption was still elevated as much as 40 per cent (20% in the 26-day-old animal represented by the graphs in fig. 1). An extreme case of such increased heat dissipation is indicated in the first quadrant of figure 2, where an oxygen consumption of 208 per cent is associated with an elevation in temperature of only 1.17°C.

The exact mechanism involved in stimulation of metabolism by ergotoxine has not been established. Githens (5) found that ergotoxine hyperthermias were diminished but not abolished by curare and that, while decapitation did abolish hyperthermia, increased muscular activity was still present. Since increased muscular activity without hyperthermia has also been observed in one of our 26-day-old rats (9), it seems that there is a metabolic stimulating effect in addition to increased muscular activity and that this is also dependent upon stimulation of hypothalamic thermoregulatory centers. Peripheral sympathetic activity could justifiably be considered as a possible additional factor in increased metabolism, but ergotoxine has been shown to be a general sympatholytic agent (3) which specifically reverses the metabolic stimulating effect of adrenalin (11). It seems probable, therefore, that hypothalamic centers may be capable of primary metabolic stimulation that is independent of muscular and peripheral sympathetic activity.

SUMMARY

Hypothermic responses to ergotoxine of young rats (in the pre-regulatory age range) were associated with decreased oxygen consumptions in one strain studied (Wistar), but a second strain (Sprague-Dawley) displayed a lack of correlation with some animals showing slight increases in metabolism. Since the correlation in the Wistar strain represents a metabolic reaction that could have occurred through the

operation of van't Hoff's Law, interference with metabolism is questionable. The hypothermias may be due to unselective stimulation of heat-production and heat-loss centers, with the latter predominating during this age range.

Hyperthermic responses in regulating rats were closely correlated with increased oxygen consumption. Since the increase in metabolic rate was approximately 2.5 times that which might be anticipated from the independent operation of van't Hoff's Law, a direct stimulation of metabolism must be responsible for it. Available evidence indicates that this metabolic stimulation is mediated through the action of ergotoxine on the hypothalamic thermoregulatory centers.

The mechanisms which may be operative in the thermal and metabolic reactions reported have been discussed.

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RELATIONSHIP OF HYPOTHERMIA TO HIGH OXYGEN POISONING^{1,2}

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THE etiology of death from exposure to high pressures of oxygen is obscure. That the metabolic level of the animal is heavily implicated has been shown by many authors using drugs known or thought to alter metabolic rate (1). Severe hypothermia, with its resultant narcosis, might be expected to offer a means of relating general metabolic level to high oxygen toxicity in the absence of complicating factors such as anesthesia, drugs, etc. The data to be presented indicate a remarkably high correlation between mortality in high oxygen pressures and oxygen consumption in rats.

METHODS

Male albino rats (Wistar strain) weighing approximately 200 grams were used throughout. They were fasted 24 hours before the experimental period and were held during the cooling phase of the experiment in tightly fitting wooden boxes especially adapted for the tail and rectal thermometer. Cooling was effected by immersion to the shoulders in 10° to 15°C. water. Temperatures were recorded by inserting a thermometer previously brought to within 1° of the expected temperature approximately 4 cm. into the rectum. Rats were cooled to 20°, 25° or 30°C. in 20 to 30 minutes and rubbed lightly with a dry towel. They were then immediately placed in the pressure chamber which consisted of a cylindrical copper tank 28 cm. in diameter and 68 cm. in length. Since no observation window was available the rats were in total darkness.

Control rats (37°) were handled in three ways; *a*) immersed in 35° to 37° water for the same duration as the hypothermic rats, *b*) dipped in water and dried with a towel to the same extent as the cooled rats and *c*) not subjected to water before being placed in the pressure chamber. No significant difference was detectable in the response of the 37° rats treated in the three ways.

Six to 10 rats per experiment were placed in the chamber and the oxygen pressure increased to 5.2 (*Series I*) or 5.8 (*Series II*) atmospheres over a period of 15 minutes. The pressure was maintained for 60 minutes and decompression to one atmosphere required 30 minutes. The chamber was continually flushed with a free flow of oxygen to prevent accumulation of CO₂. Periodic analyses of the exhaust air showed no detectable amounts of CO₂ or N₂.

Chamber temperature was maintained at 20° or 25°C. in each series. Rectal temperatures of the surviving rats were determined immediately upon removal from the chamber. Arbitrarily 10 days survival was established as the criterion of the effect of the high pressure of oxygen. It was found that in most instances rats which were severely affected died during, or within a few minutes after, the exposure. Those which survived 10 days were almost universally without symptoms.

For determination of the metabolic level a closed circuit system was used which consisted of a large bell jar seated in a ring of mercury and attached to a Sanborn respirometer. A blower main-

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² These data form a part of a thesis presented by Mr. Grossman to the Graduate School, Boston University, in partial fulfillment of requirements for the degree of Master of Arts.

tained a constant circulation of air and the CO_2 was absorbed both by the spirometer canister and by a bag of soda lime placed in the bell jar. The rats were prepared and handled exactly as described above for high oxygen exposure prior to being placed under the bell jar in groups of five. The O_2 consumption was measured over a period of 105 minutes (\approx duration of high O_2 pressure exposure). All metabolic determinations were made at one atmosphere pressure. No attempt was made to determine O_2 consumption under increased pressure. Rectal temperatures of the rats were recorded upon removal from the respiratory chamber.

RESULTS

The survival rate of rats cooled to initial rectal temperatures of 20° , 25° and 30°C . and controls (37°C .) when exposed to 5.2 atmospheres O_2 pressure for 60 minutes is shown in table 1. Table 2 shows the results of exposure of rats of the same initial temperatures to 5.8 atmospheres O_2 pressure. Each of these tables is broken

TABLE 1. SURVIVALS OF 181 RATS EXPOSED TO 5.2 ATMOSPHERES OXYGEN PRESSURE FOR ONE HOUR

INITIAL RECTAL TEMP.	CHAMBER TEMP.	SURVIVORS		INITIAL RECTAL TEMP.	CHAMBER TEMP.	SURVIVORS	
		No.	Percentage			No.	Percentage
$^\circ\text{C}$.	$^\circ\text{C}$.			$^\circ\text{C}$.	$^\circ\text{C}$.		
20	20	9 of 10	90	20	25	32 of 40	80
25	20	3 of 12	25	25	25	18 of 38	47
30	20	9 of 12	75	30	25	5 of 13	38
37	20	18 of 26	69	37	25	17 of 30	56

TABLE 2. SURVIVALS OF 140 RATS EXPOSED TO 5.8 ATMOSPHERES OXYGEN PRESSURE FOR ONE HOUR

INITIAL RECTAL TEMP.	CHAMBER TEMP.	SURVIVORS		INITIAL RECTAL TEMP.	CHAMBER TEMP.	SURVIVORS	
		No.	Percentage			No.	Percentage
$^\circ\text{C}$.	$^\circ\text{C}$.			$^\circ\text{C}$.	$^\circ\text{C}$.		
20	20	1 of 16	6	20	25	3 of 19	16
25	20	3 of 12	25	25	25	3 of 23	13
30	20	3 of 12	25	30	25	1 of 8	12
37	20	5 of 23	22	37	25	5 of 27	18

down to show the effects of varying the O_2 chamber temperature from 20° to 25°C . The difference in survival rate between these two chamber temperatures was not statistically significant (chi square = 0.79).

Table 3 shows the results of the metabolic measurement at one atmosphere pressure. Oxygen consumption is expressed as mg. O_2 /100-gm. body weight, after the method of Krantz and Carr (2). Inasmuch as it is the O_2 consumption during the one hour of exposure to the maximum pressure that is of primary concern the average figures for this period only, i.e. from 15 to 75 minutes in the metabolism chamber, are shown.

No attempt was made to maintain the hypothermic rats at a given temperature. Consequently, during the 105 minutes after the initial level was obtained the rectal temperatures progressively rose. Table 4 shows the rise in temperature of

the surviving cooled rats (both series) in both 20° and 25°C. chamber environments and at one atmosphere in the metabolism chamber. The right-hand column shows the fall in temperature of the surviving controls (37°C.) during the same exposures. Several things are shown by this table, viz. 1) the rise in temperature of the cooled rats was less and the fall in temperature of the controls was greater at 5.8 atmospheres than at 5.2°; 2) the rise of rectal temperature in the hypothermic animals was less in a 20° environment than in a 25° environment; 3) the presence of high tensions of oxygen tended to suppress the return to normal of hypothermic rats (when compared with rats cooled to the same degree but maintained at one atmosphere) and caused a fall in rectal temperature of control rats.

TABLE 3. METABOLIC RATE AT VARIOUS RECTAL TEMPERATURES AS DETERMINED AT ONE ATMOSPHERE

INITIAL RECTAL TEMP.	NO. OF RATS	AVERAGE O ₂ CONSUMPTION FROM 15 TO 75 MIN. MG. O ₂ /100-GM. BODY WT.	INITIAL RECTAL TEMP.	NO. OF RATS	AVERAGE O ₂ CONSUMPTION FROM 15 TO 75 MIN. MG. O ₂ /100-GM. BODY WT.
°C.			°C.		
20	25	89.8	30	25	187.1
25	25	198.8	37	25	162.9

TABLE 4. CHANGE IN RECTAL TEMPERATURE IN 105 MINUTES EXPOSURE TO OXYGEN AT 1, 5.2 AND 5.8 ATMOSPHERES PRESSURE

CHAMBER TEMPERATURE	CHAMBER PRESSURE, ATMOSPHERES	CHANGE IN RECTAL TEMP. FROM INITIAL TEMP. OF			
		20°C.	25°C.	30°C.	37°C.
°C.		°C.	°C.	°C.	°C.
20	5.2	+ 4.6	+ 4.0	0	-2.9
20	5.8	+ 2.6	+ 1.65	0	-7.0
25	5.2	+11.6	+ 6.7	+3.8	-2.3
25	5.8	+ 7.2	+ 6.8	+1.3	-3.0
25 to 28	1	+11.01	+11.4	+7.6	0

DISCUSSION

It is of considerable interest that the metabolic rate of rats can be first increased, then decreased, by progressively lower body temperatures. Smith and Fay (3) reported a reduction ranging from -6 to -25 per cent in metabolic rate of human subjects whose body temperatures were reduced to 23° to 32°C. for prolonged periods. Dill and Forbes (4), however, were unable to confirm this finding and reported a decrease in only 5 of their 22 observations on 9 human patients ranging from 25.5° to 38.0°C.

The latter authors point out that the oxygen consumption in hypothermia is largely a function of the degree of involuntary shivering and voluntary muscular activity. Although the rat is a notably poor shiverer, the metabolic increases noted at 30° and 25°C. rectal temperatures would suggest that muscular activity is in-

creased. Observation of the rats during the metabolic measurements did not reveal any apparent increase in voluntary muscular activity. Some rats were seen to shiver somewhat while others remained quiet. At 20°C. rectal temperature all rats were quiet and appeared to be almost completely narcotized.

It is significant that a rectal temperature of 20°C. is just 4°C. above the usual lethal temperature for rats (5). Yet none of the 25 rats cooled to this temperature for metabolic measurement succumbed. For this reason, together with the fact that overall survival of this group was the highest of any of the hypothermic rats exposed to OHP, we do not consider lethality due to the cooling in any way vitiates our results.

The most striking factor in these data is the high inverse correlation ($r = -0.96$) between metabolic level and survival of rats exposed to 5.2 atmospheres oxygen pressure. If this finding be interpreted in the light of the enzyme inhibition theory advocated by Stadie, Riggs and Haugaard (6), it can be said that metabolic enzyme susceptibility to high pressures of oxygen varies directly with the enzyme activity. It is of interest to note, however, that environmental (body) temperature of the enzymes bears no relationship to the inactivation.

The CO₂ retention theory, originally postulated by Thompson (7) appears tenable in the light of these data inasmuch as the CO₂ production, which parallels the O₂ consumption, is so highly correlated with toxicity. However, recent observations on the ability of the dog to withstand CO₂ concentrations of the order of 55% for as long as 45 minutes (8) would appear to reopen the question of CO₂ toxicity.

The sharp difference in survival rate of rats exposed to 5.2 and 5.8 atmospheres OHP is noteworthy. Apparently the latter pressure is so toxic to our rats that the beneficial effect of reduced metabolism is obscured.

Campbell (9) reported considerable protection afforded by lower environmental temperature during OHP exposure. At 24°C. he had a survival rate of 54 per cent compared with 8 per cent at 33°C. when rats were exposed to six atmospheres for 30 minutes. However, it is well known that rats do not survive well at one atmosphere in 33°C. temperature if the humidity is high. We were unable to obtain any significant difference between survival rates of rats exposed to the same pressure in 20° and 25°C.

SUMMARY

Rats cooled to rectal temperatures of 20°, 25° and 30° together with controls (37°) were subjected to 5.2 atmospheres (*Series I*) and 5.8 atmospheres (*Series II*) oxygen tension for one hour. Comparable rats were cooled in the same way and their metabolic rate determined at one atmosphere in a closed-circuit respirometer. The correlation coefficient between survival at 5.2 atmospheres and O₂ consumption was found to be -0.96 .

The oxygen consumptions of hypothermic rats were found to increase above normal at a rectal temperature of 30°, further increase at 25° and fall off sharply to below normal at 20°C. The survival rate of rats exposed to 5.8 atmospheres oxygen tension was so low as to obscure any beneficial or deleterious effects of altered metab-

olism. Altering the temperature of the pressure chamber from 20°C. to 25°C. had no significant effect on survival rate.

The return to normal temperature following cooling of the rats was delayed in the presence of high O₂ tensions over what it was at one atmosphere. Also the fall in body temperature of the control animals exposed to high O₂ tensions was greater than those at one atmosphere.

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THE THYROID AND HIGH OXYGEN POISONING IN RATS¹

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A VERY high correlation has been found to exist between the metabolic level as influenced by body temperature and susceptibility to high oxygen tension in rats (1). The beneficial effects of starvation have been pointed out by several authors (2-4) and many drugs have been administered which altered the response (5). Campbell (6) administered 0.4 mg. thyroxin to rats and found the poisoning to be enhanced. He then thyroidectomized 6 rats and found a markedly greater survival. In view of the small number of animals used and the experimental conditions involved (environmental temperature 33°C.), it was decided to extend these observations by slightly different methods.

METHODS

Male rats of approximately 200 grams were divided into three groups of 25 each. All rats received *ad libitum* the stock Rockland Farms diet. To this was added 0.03 per cent 6-n-propyl-thiouracil² for 22 days for one of the groups and 0.12 per cent desiccated thyroid powder for 8 days for the other group.² The third group served as controls. One rat in the desiccated thyroid group died on the sixth day of the diet, 2 days before exposure to the high-oxygen tension.

Equal numbers of rats from the three groups were exposed simultaneously to an oxygen pressure of 5.5 atmospheres for one hour. Fifteen minutes were required for compression and 30 minutes for decompression. The chamber was at all times flushed with a continuous flow of O₂ to prevent any accumulation of CO₂. All rats were fasted 24 hours prior to the experiment. Survival for 10 days post-exposure was arbitrarily chosen as the criterion of toxicity of the high concentration of oxygen.

Metabolic rate was checked by placing the rats in groups of five in a closed-circuit metabolism chamber at one atmosphere pressure for the same length of time as they would be in the high-oxygen chamber (105 minutes). However, since the primary concern is the metabolic rate during the 60 minutes of exposure to 5.5 atmospheres the average O₂ consumptions for this period only are shown. Oxygen consumption is expressed as mg. O₂/100-gm. body weight/hr.

RESULTS

The survival rate in the three groups of rats is shown in table 1. The significance of the differences is apparent and it is shown beyond doubt that hypothyroidism with its concomitant lowered O₂ consumption offers protection against the toxic effects of high concentrations of oxygen.

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² Dosages suggested by Dr. E. B. Astwood of the New England Medical Center, Boston (personal communication).

³ Kindly furnished by Lederle Laboratories.

DISCUSSION

The rate of cellular oxidations is apparently closely allied to the toxic effects of high concentrations of oxygen. In this series of experiments the correlation coefficient between O₂ consumption and survival in 5.5 atmospheres O₂ for one hour is -0.97 . In a previous series wherein the O₂ consumption was altered by hypothermia of the animal the correlation coefficient was -0.96 (1).

Although Bert (7) did not believe that CO₂ accumulation was an important etiological factor in high oxygen poisoning, more recent workers (5) are in essential agreement that CO₂ does accumulate within the animal in large quantities and is probably of considerable importance. These data would seem to lend further evidence for that contention since CO₂ production varies directly with oxygen consumption.

Central nervous system involvement has been recognized since the time of Bert as another etiological factor in oxygen toxicity. Precisely how the C.N.S. is dam-

TABLE 1. SURVIVAL AT 5.5 ATMOSPHERES AND O₂ CONSUMPTION AT ONE ATMOSPHERE OF HYPERTHYROID, CONTROL AND HYPOTHYROID RATS

	HYPOTHYROID (PROPYLTHIOURACIL)		CONTROL		HYPERTHYROID (DESICCATED THYROID)	
	O ₂ consumption; mg/100-gm. rat	Survivors 5.5 atmospheres	O ₂ consumption; mg/100-gm. rat	Survivors 5.5 atmospheres	O ₂ consumption; mg/100-gm. rat	Survivors 5.5 atmospheres
1	87	4 of 5	199	4 of 5	256	2 of 5
2	90	5 of 5	136	2 of 5	179	2 of 5
3	104	4 of 5	138	2 of 5	132	1 of 4
4	140	4 of 5	171	2 of 5	228	2 of 5
5	107	3 of 5	170	4 of 5	192	0 of 5
<hr/>						
	Average	Total	Average	Total	Average	Total
	106	(80%)	163	(56%)	197	(29%)

aged is not known, but the contention that it is through the accumulation of metabolic end products would appear to receive support from the results herein reported.

It is of interest to note that exposure to 5.5 atmospheres oxygen pressure for one hour closely approximates the LD₅₀ for rats of our strain and age. In this series at 5.5 atmospheres 14 of 25 control rats survived (56%) whereas in two previous series 35 of 56 (62%) control rats survived one hour of 5.2 atmospheres and only 10 of 50 (20%) survived exposure of one hour to 5.8 atmospheres O₂ pressure (1).

SUMMARY

Seventy-five rats of approximately 200 grams each were divided equally into three groups. All were fed a stock diet to which was added propylthiouracil (22 days) for one group and desiccated thyroid powder (8 days) for the second group. The third group served as controls. Oxygen consumptions were determined at one atmosphere pressure after which the animals were exposed to 5.5 atmospheres O₂ pressure for one hour. The correlation coefficient between oxygen consumption

and survival rate in the three groups was -0.97 , a highly significant figure. It is suggested that CO_2 production and accumulation is an important factor in the etiology of high oxygen poisoning.

Five and one half atmospheres O_2 pressure for one hour is considered to be the approximate LD_{50} for our strain and age rats.

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EFFECT OF VARIOUS NERVE LESIONS UPON THE BLOOD FLOW TO THE HIND EXTREMITY OF THE DOG¹

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IT HAS been a general clinical impression that the blood supply to a paralyzed extremity undergoes a significant reduction. Solandt (1) has suggested that this postulated decrease may contribute greatly to the atrophy of denervation. Harris and McDonald (2) believe that the poor growth of limbs, which have been affected by anterior poliomyelitis, is largely due to an inadequate circulation to the extremity. These authors have removed the lumbar sympathetic chain in such patients and have claimed beneficial effects upon limb growth in those patients who responded with a sustained increase in circulation. Telford and Stopford (3) have reported a small number of cases in which a lumbar sympathectomy has relieved the distress caused by an increased sensitivity to cold of extremities paralyzed by anterior poliomyelitis.

However, clinical studies on the effect of denervation upon blood flow have revealed no significant differences in resting flow between the normal and paralyzed limbs (4-6). These findings contrast with those of Kemp, Tuttle and Hines (7) who observed that, in the dog, chronic denervation of a hind leg resulted in a significant decrease of both its blood flow and deep tissue temperature.

The present investigation was carried out in order to obtain additional information concerning the rôle of the various components of the peripheral nervous system in the vascular responses of the paralyzed extremity of the experimental animal.

METHODS AND MATERIALS

Three groups of dogs were prepared with unilateral nerve lesions of a hind extremity as follows. *a) Ventral group:* 14 animals; section of ventral roots L₄ to L₇ inclusive. *b) Dorsal-ventral group:* 9 animals; section of dorsal and ventral roots L₄ to L₇ inclusive. *c) Peripheral nerve group:* 12 animals; section of the sciatic femoral and obturator nerves at their emergence from their respective foramina.

The choice of location of these lesions was based upon the following experimental facts. There is good agreement that in a variety of animals the major sympathetic outflow to the hind legs occurs between T₁₃ and L₂, with an occasional contribution from L₄ (see 8 for review of earlier literature, 9). In the dog, the major motor outflow to the muscles of the lower leg occurs between L₄ and L₇ (10, 11). Thus, rhizotomies involving these segments can produce a complete paralysis of these muscle groups with a minimum of interference with the sympathetic innervation. In actual practice these lesions also produced a considerable paralysis of the muscles of the upper leg and so, essentially, the animals had flail limbs. Finally, since all available evidence (12-15) indicates that the sympathetics

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to an extremity are contained in its mixed peripheral nerves, appropriate peripheral nerve section should effect a total denervation of the limb.

These three groups of animals, then, represented the following types of motor disability: *a) Ventral group*: loss of somatic motor innervation alone. *b) Dorsal-ventral group*: a combined motor and sensory loss. *c) Peripheral group*: loss of both somatic and sympathetic innervation.

One week pre-operatively and at intervals of 2, 4 and 8 weeks post-operatively, temperatures of the rectum, of both gastrocnemii and of the subcutaneous tissue over the dorsum of both feet were taken by means of iron constantan-copper thermocouples. The temperatures were read with a Leeds Northrup potentiometer whose values were calibrated for each couple individually. Measurements were made every 15 minutes for one hour. The animals were maintained under pentobarbital anesthesia at a room temperature of approximately 30° C. Preliminary experiments had demonstrated that at lower room temperatures the rectal temperature and peripheral blood flow tended to drop rapidly.

At the end of 12 weeks the blood flow in both femoral arteries was determined by means of the bubble flow meter (16). Clotting of the blood was prevented by an oral dose of 100 mg. of dicoumarol 24 to 48 hours before the experiment and by 100 mg. of heparin intravenously just before the necessary cannulation of the arteries. The thermocouples were set in place in the manner described above. Temperature and flow readings were begun 20 minutes after the arteries were connected to the flow meter. Three successive bilateral flow readings were taken simultaneously every 10 minutes and temperature measurements every 20 minutes over a one-hour period.

Prior to death the extent of the motor deficit was ascertained by electrical stimulation of the sciatic and femoral nerves. Such stimulation revealed only trace responses of the quadriceps (6 dogs), hamstrings (5 dogs) and toe flexors (12 dogs) of the ventral and dorsal-ventral groups only. Immediately after the animal was killed, both hind legs were amputated at the knee joint by means of an ordinary band saw. The legs were weighed and then the muscles were dissected free and weighed separately.

Twenty normal dogs served as a control group to evaluate the fluctuations in blood flow through the femoral arteries in the same animal. Statistical analysis of all data was carried out by the method of paired comparisons.

RESULTS

A necessary preliminary to the use of any experimental method should be an estimation of its reliability. For this purpose, the blood flow data obtained from the control group of normal animals were treated by an analysis of variance (table 1). The information obtained by this analysis indicates that 1) the three consecutive readings in the same 10-minute period are in good agreement (*reliability coefficient* = .98); 2) the method is capable of detecting differences between animals ($F = 8.6$); 3) there is a significant difference between the flow readings at the successive 10-minute intervals ($F = 8.6$). Further examination of the data indicates that this difference is due to a progressive decrease of blood flow of approximately 20 per cent during the hour period. This decrease could not be correlated with changes in rec-

tal, muscle or subcutaneous temperatures. 4) There are no significant differences between the flows to the right and left legs ($F = .8$). The tendency for the blood flow to fall off during the period of observation does not invalidate this method since this trend affects both legs in the same direction and to the same degree. The bubble flow meter when used under the conditions described above, is a reliable procedure for the measurement of blood flow.

A. *Temperature Studies* (table 2). In the *normal group* there were no significant differences in mean subcutaneous and muscle temperatures between the right and left legs. There was a good correlation of temperature between the legs of the same animal.

In the *ventral* and *dorsal-ventral groups* no significant differences in mean subcutaneous and muscle temperatures were observed, 2, 4, 8 or 12 weeks post-opera-

TABLE 1
A. Reliability of Blood Flow Determinations

NO. OF DOGS	AVERAGED BLOOD FLOWS (CC/MIN.) OF RIGHT AND LEFT FEMORAL ARTERIES. READINGS TAKEN AT 10-MIN. INTERVALS FOR 1 HR.						
	0 min.	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
19	63.4	60.6	58.0	56.1	54.9	53.3	51.6

B. Analysis of Variance

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARES	F RATIO
Dogs.....	18	655.0	8.6 ¹
Readings at 10-min. intervals.....	6	653.7	8.6 ¹
Right and left legs.....	1	61.5	.8
Error.....	240	76.0	

Intraclass correlation = $\frac{6550}{6550 + 76} = .99$ (Used as measure of reliability). ¹ Significant at 1% level.

tively between the normal and denervated limb. Limb temperatures of the same animal corresponded closely.

In the *peripheral nerve group* significant decreases in the subcutaneous and muscle temperatures of the denervated leg were observed at the 4th and at the 12th week, respectively.

B. *Blood-Flow Studies* (table 3). In the *normal group* the volume flow in the right and left femoral arteries was essentially equal in the same animal (*mean correlation* = .97).

In the *ventral* and *dorsal-ventral groups* no significant differences were observed in the mean femoral artery blood flow between normal and denervated legs.

In the *peripheral nerve group* the mean flow through the femoral artery of the denervated limb was 17.7 per cent less than that of the normal side. This decrease is significant at the 5 per cent level of probability.

C. *Leg and Muscle Weights* (table 4). The mean weights of right and left legs

are essentially the same in the normal dogs. In the individual animals the weights of left and right legs are closely correlated. Significant losses of both leg and muscle weight occurred in the denervated extremities of all three groups. The decrease in leg weight can be accounted for almost entirely by the loss of muscle mass. A com-

TABLE 2. TEMPERATURE STUDIES

GROUP	WKS. POST-OPER.	NO. OF DOGS	MEAN SURCUT. TEMP. (°C.)					MEAN MUSCLE TEMP. (°C.)					RECTAL TEMP. (°C.)
			Den. side Rt.	Norm. side Lt.	Diff. °C.	t ratio	Corr. coeff.	Den. side Rt.	Norm. side Lt.	Diff. °C.	t ratio	Corr. coeff.	
Controls		22	37.3	37.2	.1	.72	.70	38.3	38.3	0	.76	.97	39.1
Ventral	2	6	36.8	37.3	-.5	1.48	.88	38.0	38.1	-.1	1.72	.87	38.2
	4	8	37.3	37.5	-.2	.80	.94	38.1	38.2	-.1	1.33	.88	38.4
	8	10	37.5	37.3	+.2	1.15	.76	38.0	38.1	-.1	1.27	.94	39.1
	12	10	37.0	36.9	+.1	.55	.77	37.2	37.2	0	.29	.92	39.1
Dorsal - ventral	2	6	37.8	38.1	-.3	1.02	.89	38.7	38.7	0	.27	.90	39.1
	4	8	37.2	37.4	-.2	.90	.85	38.0	38.1	-.1	.77	.95	38.5
	8	8	37.1	37.2	-.1	.39	.54	37.8	37.9	-.1	.84	.58	38.6
	12	9	37.7	37.6	+.1	.32	.93	38.4	38.3	+.1	.38	.89	39.5
Peripheral	2	9	37.2	37.8	-.6	1.72	.90	38.2	38.6	-.4	2.10	.88½	39.5
	4	8	36.8	37.6	-.8	2.82¹	.79	38.1	38.3	-.2	1.72	.88	38.9
	8	8	37.4	37.6	-.2	1.18	.93	37.9	38.0	-.1	1.29	.96	39.1
	12	9	36.2	37.6	-1.4	2.76¹	.84	37.9	38.3	-.4	3.03²	.82	39.5

Each temperature reading represents the combined mean of 5 determinations over a 1-hr. period for the number of dogs indicated.

¹ Significant at 5% level. ² Significant at 2% level.

TABLE 3. BLOOD FLOW

GROUP	NO. OF DOGS	MEAN FLOW (CC/MIN.)		t RATIO	CORREL. COEFF.
		Normal leg	Denervated leg		
		Rt. leg	Lt. leg		
Normal animals.....	20	60.0	58.5	.52	.97
Ventral root section.....	14	45.7	44.4	.51	.86
Dorsal and ventral root section.....	9	49.7	48.2	.89	.99
Peripheral nerve section.....	12	43.5	35.8	2.21¹	.85

¹ Significant at 5% level of probability.

parison of the loss of muscle weight which occurred in the three groups indicates that the degree of muscle atrophy was the same for all three.

DISCUSSION

The results of these experiments suggest that, in the dog, the resting blood flow to a denervated limb is essentially unchanged so long as the sympathetic outflow to

the limb is intact. The significantly lower temperatures and blood flows of the denervated limb following total (peripheral) nerve section must be considered against the background of the experimental conditions. The room temperature, maintained at 30° C., represents a warm environment; and if vasodilatation in the extremities of the dog occurs in warm environments as it does in man, this state should be favored in the intact limb. In addition, the anesthesia with its depressing effect upon the central nervous system may have produced a diminution of sympathetic vascular tone. Therefore, the warm environment and anesthesia which may increase the blood flow to the normal side may not be expected to produce similar vascular effects upon the denervated side.

Kemp, Tuttle and Hines (7) have suggested that the decreased blood flows which they observed in the peripherally denervated limbs of dogs may be due to 1) an increased sensitivity of the denervated vessels to vasoconstricting agents present in the blood and 2) a deficiency of vasodilatation mechanisms in the extremity. In regard to this latter point, there is some evidence for the existence of vasodilator fibers to the limb muscles of the dog and cat (17, 18), although the functional significance of

TABLE 4. LEG AND MUSCLE WEIGHTS

GROUP	NO. OF ANIMALS	MEAN LEG WEIGHT (GM.)		I RATIO	CORR. COEFF.	MEAN MUSCLE WEIGHT (GM.)		I RATIO	CORR. COEFF.
		Normal	Denerv.			Normal	Denerv.		
		Right	Left						
Normal.....	20	380	381	.43	.98				
Ventral.....	14	324	275	9.03 ¹	.92	93.3	52.1	10.3 ¹	.69
Dorsal-ventral.....	9	364	310	5.45 ¹	.40	97.7	50.7	8.04 ¹	.49
Peripheral.....	12	378	319	6.93 ¹	.65	114.6	58.0	7.36 ¹	.40

¹ Significant at the 1% level of probability.

these fibers is as yet obscure. Siems, Kosman and Osborne (19) have recently demonstrated in the dog that the typical response of vasodilatation which occurs in a limb subjected to local heating may depend upon the integrity of its sympathetic supply.

The failure of the ventral and dorsal-ventral groups to exhibit the flow and temperature changes shown by the peripherally denervated animals would indicate that the loss of motor function and of muscle weight per se does not result in any reduction of resting blood flow to the limb under the conditions of the experiment. In this respect these animal experiments are in agreement with the clinical studies of Abramson *et al.* (5) in patients suffering from anterior poliomyelitis. Although, conventionally, blood flows are recorded in terms of unit limb volumes or weights, we did not follow this convention because the correlation between leg weight and blood flow in normal animals is exceedingly poor ($r = -.23$). We considered it pointless to express blood flow in terms of a factor with which it may have little or no relationship.

It may be added that our observations on the loss of muscle weight resulting from the three types of nerve lesions offer additional experimental support to the con-

clusion of Tower (20) that the loss of motor innervation is the only nervous factor directly involved in the atrophy of the contractile portion of skeletal muscle which follows total denervation.

SUMMARY

Unilateral nerve lesions were produced in the hind limbs of 35 dogs, falling into three groups. a) Ventral root section, L₁ to L₇, inclusive. b) Combined dorsal and ventral root sections, L₁ to L₇, inclusive. c) Peripheral nerve section. All dogs showed an essentially complete motor paralysis of the hind extremity.

Twelve weeks after the production of the initial lesion, no significant differences in femoral artery blood flow were observed between the normal limb and the limb denervated by either a ventral or dorsal-ventral root lesion. A significant reduction in flow occurred, however, in those extremities subjected to a peripheral nerve section. It is suggested that the reduced blood flow which may be present in a peripheral nerve paralysis is due to involvement of the sympathetic supply.

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OBJECTIVE EVALUATION OF TRANSFUSION THERAPY IN HEMORRHAGIC SHOCK^{1,2}

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RECENT work from this laboratory has shown that when the blood volume of an unanesthetized dog is suddenly reduced by a massive hemorrhage, the cardiac output is markedly decreased (1). This slowing of the blood flow soon leads to major disturbances in the blood chemistry and acid-base balance. The most striking are the decreases in arterial pH and CO_2 content and the increases in the concentrations of lactate and phosphate (2). This work suggested that measurement of the changes in certain blood constituents would provide an index not only of the depth or degree of shock, but also for testing, at various stages, the effectiveness of replacement therapy. The development of a standard procedure for the production of hemorrhagic shock (3) has made it possible to examine critically some of the criteria used in evaluating the physiological condition of an animal and to study the effect of transfusion with blood or plasma during the terminal stages of hemorrhagic shock.

PROCEDURE

Only healthy adult mongrel dogs were used. The animals had access to water but were not fed for 24 hours before experimentation. In each experiment the dog was placed upon its back on an animal board, a control blood volume determination was made, and the bladder emptied through an urethral catheter. After exposure of both femoral arteries under local anesthesia (procaine hydrochloride with subsequent infiltration with neothisol⁵) one artery was cannulated and the control mean arterial blood pressure measured with a mercury manometer. Blood samples were drawn anaerobically from the opposite femoral artery via a small musculo-cutaneous branch for control pH , arterial O_2 and CO_2 content, whole blood lactate, plasma inorganic phosphate and plasma amino nitrogen. At the same time jugular venous blood was drawn for the determination of O_2 content. After these samples had been collected, the animal was bled rapidly through the femoral arterial cannula until the flow had

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⁵ Two parts methyl methylene para amino phenyl formate and 5 parts hydroxybenzocarbeneal in refined almond oil. Caso Laboratories, New York City.

practically ceased. Twenty-five per cent of the bleeding volume which had been collected in syringes previously rinsed with heparin solution was returned to the animal through the arterial cannula (3). The remaining blood was prevented from clotting by liquid heparin or citrate and kept at room temperature. The femoral cannula was then reconnected with the mercury manometer for the continuous recording of blood pressure. Arterial blood samples for the various chemical analyses were taken every half hour after hemorrhage and the blood volume was re-determined one hour after bleeding.

When the mean arterial blood pressure had fallen to 25 mm. Hg or less and was still falling, the animal was transfused with whole blood or plasma. We have used the word 'terminal' to refer to the time at which transfusion was carried out. Sometimes the terminal stage was reached so rapidly that respiration ceased and blood pressure fell to almost zero before transfusion was started. Four dogs were transfused through the femoral vein, the others through the femoral artery. The blood removed during the bleeding was used for whole blood transfusion. For plasma transfusion, the blood removed by hemorrhage was centrifuged and the plasma obtained was added to enough reconstituted, pooled, lyophilized canine plasma to make a quantity equivalent to 75 per cent of the bleeding volume.

After transfusion blood samples for the various analyses were taken every hour for 4 hours. A third blood volume determination was made 2 hours after volume replacement. Blood pressure measurements were continued, heart rates counted and the rate of urine excretion determined. Four hours after transfusion the incisions in both thighs were treated with sulfathiazole, closed with sutures and the animal was placed in a cage and allowed to drink water freely.

METHODS

The methods used for the determination of blood volume, blood pH, the CO₂ content of whole blood or plasma, whole blood lactate and plasma inorganic phosphate were those previously used in this laboratory (2). The O₂ content of the blood was estimated by the microgasometric method of Roughton and Scholander (4). Plasma amino nitrogen was measured gasometrically using ninhydrin-CO₂ methods according to Hamilton and Van Slyke (5).

RESULTS

Twenty-one dogs in experimental hemorrhagic shock were transfused at the terminal stage with their own whole blood. Sixty-two per cent of the animals survived; the others succumbed within 24 hours of the transfusion. In a second series 16 dogs were transfused in the terminal stage with plasma. Eighty-one per cent of these animals survived, only 3 dogs dying within 24 hours. The data have been averaged for the four groups, namely: whole blood survivors and nonsurvivors and plasma survivors and non-survivors.

Blood volume. The control blood volumes varied from 63 to 108 cc kg., the average being 92.5 cc. Table 1 shows that there was no significant difference between the control blood volumes for the groups of whole blood and plasma transfused survivors and non-survivors. The terminal blood volumes (i.e. blood volumes measured one hour after hemorrhage minus all the subsequent samples taken for analyses) of all groups fell between 57 and 63 per cent of the control volumes. The

fact that the terminal blood volume was greater than the volume calculated from the control volume minus the blood removed by bleeding and for analyses shows that compensatory dilution occurred. This is also reflected by the decrease in plasma protein concentration which averaged 17 per cent (table 1). In spite of hemodilution, transfusion with either whole blood or plasma did not completely restore blood volumes to the control values. This can be explained in part by the volume of blood taken for the various analyses.

Practically all of the animals exhibited diarrhea in varying degrees during some part of the post-hemorrhagic period. The diarrhea was most apparent after transfusion and in a considerable number of the dogs the stools were bloody. Autopsy of the non-surviving animals showed intestines filled with fluid. That fluid was lost

TABLE 1. EFFECT OF PLASMA AND WHOLE BLOOD TRANSFUSIONS UPON AVERAGE CHEMICAL CHANGES PRODUCED IN THE BLOOD BY HEMORRHAGE

	WHOLE BLOOD						PLASMA					
	Survivors— n = 13			Non-survivors— n = 8			Survivors— n = 13			Non-survivors— n = 3		
	C	T	P	C	T	P ¹	C	T	P	C	T	P
Blood vol. cc/kg.....	92.3	57.8	83.7	89.8	53.7	80.7	96.4	55.4	76.1	97.6	57.6	80.3
Hematocrit.....	44.1	40.5	46.8	44.0	43.1	51.8	45.8	42.1	25.3	46.8	47.2	29.3
Plasma protein gm. %.....	5.5	4.4	5.4	5.9	4.9	6.1	5.9	4.9	6.3	5.7	5.4	6.0
pH.....	7.35	7.14	7.35	7.36	7.06	7.28	7.36	7.17	7.41	7.38	7.16	7.22
Art. CO ₂ content vol. %.....	40.7	16.4	33.2	42.2	10.2	22.9	41.6	13.5	38.3	43.3	8.9	28.0
Art. O ₂ content vol. %.....	18.0	16.5	19.5	17.1	18.0	19.7	18.3	17.0	10.3	18.2	19.0	12.4
Venous O ₂ content vol. %.....	12.2	2.0	10.0	12.5	3.3	5.3	13.4	1.8	4.2	12.9	1.7	2.9
A-V O ₂ diff. vol. %.....	5.5	14.6	9.2	4.5	13.9	14.6	4.4	15.3	6.2	5.3	17.3	8.5
Whole blood lactate mg. % ²	23.4	113.2	19.5	22.3	141.5	52.5	21.4	143.4	16.8			
Plasma inorg. P mg. % ²	3.7	14.3	7.7	3.3	14.9	9.9	4.4	15.1	8.1			
Plasma amino N mg. % ²	3.85	6.95	5.04	3.40	7.36	6.80	3.56	7.00	4.34			

n = number of animals; C = control; T = terminal; P = 4 hours post-transfusion.

¹ Samples taken immediately before death varied from 1 to 4 hours after transfusion.

² Values given are averages of determinations made on blood of 7 whole blood survivors, 6 whole blood non-survivors and 6 plasma survivors.

from the circulation is suggested by the increased hematocrit values found after transfusion in the dogs receiving whole blood (table 1).

Blood pressure. Immediately after a 75 per cent hemorrhage the mean arterial pressure was usually about 30 mm. Hg. It gradually increased to between 50 and 60 mm. Hg as hemodilution and other compensatory processes occurred and then slowly decreased. When fluid was infused the pressure returned rapidly toward the control level. The average value to which the blood pressure returned immediately after volume replacement as well as the average level maintained thereafter was definitely lower in the animals which died than in the corresponding group of animals that survived. Four hours after transfusion with whole blood, the average mean arterial pressure of the surviving animals was 102 mm. Hg. This happens to be identical with the average value obtained on the same dogs during the control period.

In the dogs which received whole blood but did not survive, the average blood pressure at 4 hours was 64 mm. Hg, the average control mean arterial pressure in the same animals was 111 mm. Hg. The average blood pressure 4 hours after volume replacement with plasma was 90 mm. Hg (control 107) in the surviving group and 46 mm. Hg (control 107) in the group that did not survive.

Heart rate. After hemorrhage the heart rate increased from an average control value of about 90 to approximately 200 beats per minute. The rate decreased immediately after transfusion with either plasma or whole blood to about 175 and then slowly increased during the next 3 or 4 hours (190-225). During this period there seemed to be no significant difference in heart rates between the survivors and non-survivors.

Hematocrit value. In nearly all of the animals hemorrhage was followed by a decrease in the hematocrit readings (table 1). After whole blood infusion the hematocrit values returned to, or rose slightly above, the control level. Plasma transfusion, of course, diluted the red cells and lowered the hematocrit readings considerably (range 25 to 29).

Venous O₂ content (fig. 1). The average control jugular venous O₂ content was 13 volumes per cent. After hemorrhage this decreased, the terminal values being as low as one volume per cent in some instances. In dogs which survived after receiving whole blood transfusion, the average jugular venous O₂ content increased to 10 volumes per cent one hour after volume replacement. Thereafter, the venous O₂ content rose slowly toward the control values. The increase in jugular venous O₂ content was less in dogs which were transfused with whole blood but died within 24 hours. In these the venous O₂ content rose on the average to only 7 volumes per cent one hour after transfusion and then fell until death occurred. Plasma transfusion diluted the circulating red cells and in this series the jugular venous O₂ content rose to only 4 volumes per cent one hour after volume replacement. On the basis of jugular venous O₂ content it was not possible to distinguish surviving from non-surviving animals until 4 hours after plasma transfusion, after which time the venous O₂ content of non-surviving dogs slowly decreased.

A-V O₂ difference. The control arterial-jugular venous O₂ difference of the various groups of animals varied from 4.4 to 5.5 volumes per cent. After hemorrhage these values increased, ranging between 13.9 and 17.3 volumes per cent. Four hours after transfusion the average arterial-jugular venous O₂ difference of the dogs which were permanently resuscitated by whole blood transfusions was 9.2 volumes per cent, whereas the value for those which received whole blood but died was 14.6 volumes per cent. The values for the corresponding plasma transfused groups were 6.2 and 8.5 volumes per cent, respectively.

Phosphorus. The control plasma inorganic phosphorus averaged 3.5 mg. per cent. After bleeding and immediately before transfusion the average value had increased to 14.7 mg. per cent. Two hours after plasma or whole blood transfusion the average inorganic phosphorus of permanently resuscitated animals had decreased to 8 mg. per cent at which level it was maintained for the next 2 hours. In the dogs in which whole blood transfusion did not bring about permanent resuscitation, the average inorganic phosphorus fell to 9 mg. per cent 2 hours after volume replacement, then increased to about 10 mg. per cent in the succeeding 2 hours.

Lactate. The average control whole blood lactate was 22.4 mg. per cent. After hemorrhage and immediately before transfusion the average lactate concentration had increased to more than 100 mg. per cent. The terminal lactate concentration was higher in animals in which transfusion with whole blood did not produce permanent recovery than in surviving dogs. When whole blood transfusion brought about permanent recovery the whole blood lactate decreased rapidly to about 20 mg. per cent 4 hours after volume replacement. On the other hand, the lactate values decreased slowly and never fell below approximately 50 mg. per cent in the dogs which

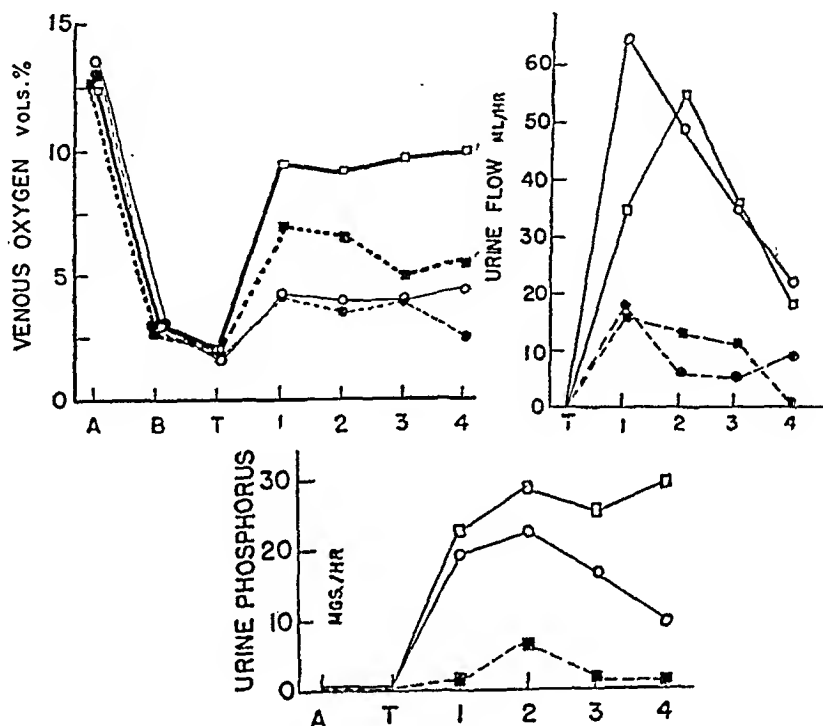


Fig. 1 (upper left). AVERAGE CHANGES in jugular venous O_2 content which occur after hemorrhage and transfusion. Abscissa: A = control jugular venous O_2 content; B = jugular venous O_2 content measured at a time equi-distant between hemorrhage and transfusion; T = terminal point, i.e. immediately before transfusion; 1, 2, 3, etc. = hours after transfusion. Dogs hemorrhaged and transfused: O = with plasma—survived; ● = with plasma—died; □ = with whole blood—survived; ■ = with whole blood—died.

Fig. 2 (upper right). EFFECT OF TRANSFUSION upon the urine flow of hemorrhaged dogs. Letters, figures, and symbols as in fig. 1.

Fig. 3 (lower). EFFECT OF TRANSFUSION upon the urine phosphate of hemorrhaged dogs. Letters, figures, and symbols as in fig. 1.

died after whole blood transfusion. The results obtained after plasma transfusion cannot be quantitatively compared with the determinations made after whole blood transfusion because of differences in the hematocrit values. However, after plasma transfusions the whole blood lactate concentration of the dogs which lived fell to about 17 mg. per cent in the subsequent 4 hours.

The pH. The pH of the arterial blood of 34 dogs, measured during the control period, varied from 7.31 to 7.40, averaging 7.36. After hemorrhage the pH decreased and immediately before transfusion it ranged in the different groups between 7.06 and 7.17. Replacement of the blood volume lost with either plasma or whole blood increased the average pH within one hour to 7.28 and 7.33, respectively. Four hours

after transfusion the pH of the arterial blood had returned to the control values in the dogs in which volume replacement with either plasma or whole blood brought about permanent resuscitation, whereas in animals in which transfusion did not prevent death the arterial pH was 0.08 to 0.16 below the control values.

Amino N. After hemorrhage the plasma amino nitrogen concentration increased from an average control of 3.6 to an average terminal value of about 7 mg. per cent. Four hours after plasma or whole blood transfusions which brought about permanent recovery the plasma amino nitrogens were 4 to 5 mg. per cent. In dogs which died after volume replacement with whole blood the plasma amino nitrogen was 6.4 mg. per cent one hour after transfusion and 2 hours later had increased to 6.8 mg. per cent.

Bicarbonate. The plasma bicarbonate concentration was calculated from the plasma CO_2 content and the pH of the arterial blood (2). During the control periods the plasma bicarbonate ranged between 18.4 and 23.5, averaging 20.8 mm. per liter.

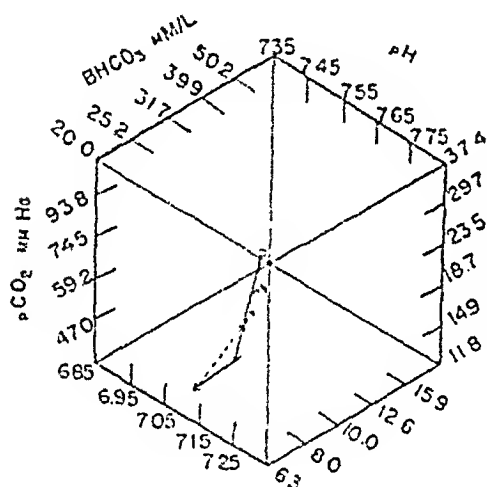


Fig. 4. ACID-BASE CHANGES in 13 dogs in which whole blood transfusions brought about permanent recovery. The solid line arising from the circle and ending in the first arrow describes the development of acidosis from the control to a time equidistant between hemorrhage and transfusion. The solid line beginning at this point and ending in the second arrow indicated the path of acidosis to the time of transfusion. Recovery from acidosis is shown by the dotted line. On this line arrows indicate the recovery which has occurred 1, 2, 3, and 4 hours after transfusion.

After hemorrhage and immediately before transfusion the individual values varied from 2.7 to 13.8 mm. per liter. The average terminal value for the dogs which were permanently resuscitated by whole blood transfusion was 8.0, and that for animals which died in spite of volume replacement with whole blood, was 4.9 mm. per liter. The corresponding figures for the plasma series were 6.5 and 4.4. Four hours after transfusion the average bicarbonate level was 16.4 mm. per liter in dogs which survived as a result of whole blood transfusion, whereas it was only 10.5 in animals which, in spite of volume replacement with whole blood, died. The corresponding values for the plasma-treated animals were 18.7 and 10.4 mm. per liter, respectively. Our results show that all the dogs in which volume replacement did not bring about permanent recovery had a terminal plasma bicarbonate concentration of 6 mm. or less per liter of plasma. When the terminal plasma bicarbonate reached these low levels permanent resuscitation was brought about in only 2 of 10 dogs by whole blood transfusion and in 8 out of 11 dogs by plasma transfusion. In no instance did transfusion with either plasma or whole blood produce recovery when the plasma bicarbonate value was less than 14 mm. per liter 4 hours after volume replacement. On the

basis of the bicarbonate data there appears to be a sharp demarcation between dogs in which volume replacement resulted in recovery and those in which the therapy failed

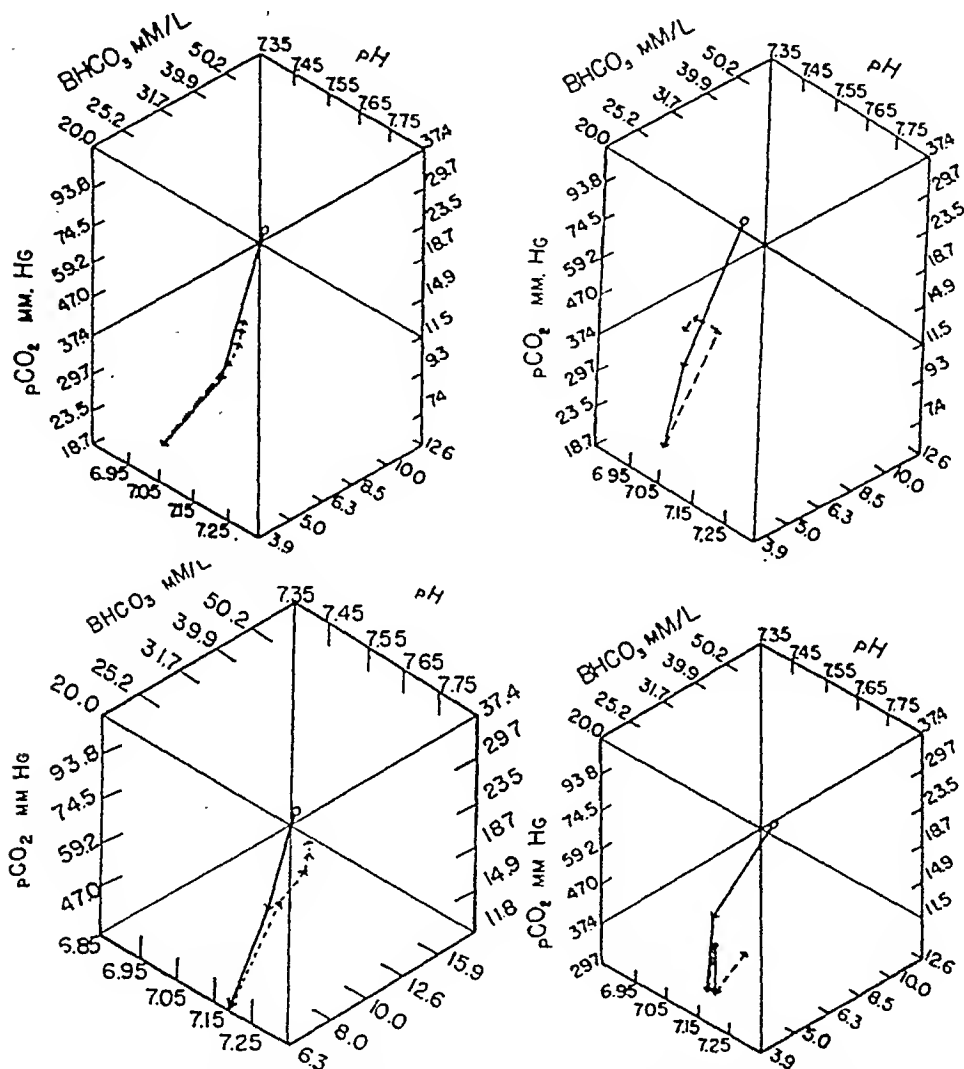


Fig. 5 (*upper left*). ACID-BASE CHANGES in 8 dogs which died in spite of whole blood transfusions. The values given are the averages of the results obtained on 8 dogs which died. The lines are drawn according to the plan described under fig. 4.

Fig. 6 (*lower left*). ACID-BASE CHANGES in 13 dogs in which plasma transfusions brought about permanent recovery. The lines are drawn according to the plan described under fig. 4.

Fig. 7 (*upper right*). ACID-BASE CHANGES in 3 dogs which died in spite of plasma transfusions. The lines are drawn according to the plan described under fig. 4.

Fig. 8 (*lower right*). TYPICAL CURVE of the acid-base changes after hemorrhage and saline infusion in one dog. The first solid line arising from the control circle indicates the development of acidosis following hemorrhage. The dotted line originating at the end of this solid line shows the path of recovery after saline infusion. Within an hour recovery ceased and a severe terminal acidotic state developed as shown by the second solid line. The second dotted line indicates the recovery after a second saline infusion. The dog died a few hours later.

Urinary secretion. After hemorrhage urinary secretion ceased or decreased to low levels, as has been reported from this and other laboratories (2). Transfusion with either plasma or whole blood was uniformly followed by diuresis. Dogs in

which transfusion produced permanent recovery showed a greater urine flow than did those which died within 24 hours of the transfusion (fig. 2). Animals in which volume replacement with plasma resulted in permanent resuscitation excreted an average of 65 cc. of urine during the first hour after transfusion. In the next 3 hours the rate of excretion fell steadily to 22 cc. per hour. Dogs which survived as the result of whole blood transfusion produced on the average 55 cc. of urine per hour for 2 hours after volume replacement. During the succeeding 3 hours the urine flow fell to 18 cc. per hour. On the other hand, the urine flow in dogs in which transfusion with plasma or whole blood failed to produce permanent recovery from hemorrhage was low, reaching only about 17 cc. during the first hour after volume replacement and decreasing to less than 10 cc. per hour at the end of 4 hours.

When hemorrhage was followed by whole blood transfusion the urinary phosphorus of the dogs in which permanent recovery occurred increased rapidly, reaching an average maximum of 29 mg. per hour (fig. 3). This was maintained for 4 hours. A similar phosphaturia was present in the animals in which plasma transfusion brought about recovery (fig. 3). In these dogs the post-transfusion urinary inorganic phosphate reached a maximum of 22 mg. per hour 2 hours after volume replacement and then fell to 9 mg. per hour 2 hours later. Little or no phosphaturia developed in dogs which died within 24 hours of plasma or whole blood transfusion.

Acid-base balance. The nature of the changes in acid-base balance after hemorrhage and transfusion has been shown by plotting the pH , the pCO_2 and the plasma bicarbonate concentration of the arterial blood on triangular co-ordinate paper (8). The average curves for dogs in which hemorrhage was followed by blood transfusion are shown in figures 4 and 5. The corresponding curves illustrating the effect of plasma transfusion appear in figures 6 and 7. In confirmation of previous work done in this laboratory (2), it will be seen that after hemorrhage all of the curves fall in the sector of metabolic acidosis, partly compensated by a decrease in pCO_2 . The severity of the terminal acidosis was greater in dogs in which whole blood transfusion did not produce recovery (fig. 5) than in those in which such replacement therapy resulted in permanent resuscitation (fig. 4). Also in the former, recovery from the acidosis was not as complete 4 hours after transfusion as in the latter group. The same results were shown by the animals in which plasma transfusion was carried out (figs. 6 and 7).

Four dogs not included in table 1 were given in the terminal stage saline infusions equal in amount to the blood removed by bleeding. All of these animals died within 4 hours of the infusion. Figure 8 illustrates the results obtained in a representative experiment. The graph demonstrates the small extent and the transitory nature of the acid-base recovery which occurs when volume replacement is attempted with saline.

DISCUSSION

Effects of transfusion. One of the most dramatic effects of transfusion is the rapid return of blood pressure to control levels. Our results, however, indicate that the restoration of blood pressure is not in itself a reliable index of the physiological condition of the animal, for non-surviving dogs may have a high post-transfusion blood pressure for several hours and yet show little or no recovery as measured by

other criteria. None of the dogs recovered immediately from the lethargy which was invariably present at the time of transfusion. The surviving animals, however, gradually became more alert and, except for an unnatural quietness, appeared reasonably normal 4 hours after transfusion.

Comparison of results shown by dogs permanently resuscitated by transfusion with those obtained on animals which died after volume replacement. The jugular venous O_2 content, the arterial pH , and the concentrations of arterial plasma bicarbonate, plasma inorganic phosphorus and whole blood lactate of hemorrhaged dogs in which plasma or whole blood transfusion results in permanent recovery return toward the control values within 4 hours of the time of volume replacement. During this period the recovery of the various blood constituents is much less in animals which die within 24 hours of transfusion.

Too much reliance must not be placed upon the absolute figures which we have given for the arterial plasma bicarbonate concentrations as a means of distinguishing dogs in which transfusion will produce recovery from those which die soon after volume replacement, for they do not appear to be valid when applied to a more recent series of experiments carried out in this laboratory (10), in which the dogs were carefully selected and fed a diet calculated to increase their vitamin and protein reserves. The degree of metabolic disturbance that animals will tolerate probably depends to some extent on their physiological condition. Additional evidence for this view has been obtained in a study of the sensitivity to morphine shown by dogs recovering from hemorrhage (11).

The most striking difference between dogs which were permanently resuscitated by whole blood or plasma transfusions and those which died after volume replacement is revealed by the volume of urine flow (fig. 2) and the rate of urine phosphate excretion (fig. 3). The large excretion of water and phosphate in dogs in which volume replacement resulted in permanent resuscitation indicates that in these animals the circulatory recovery was great enough to permit the kidney to play its usual rôle in correcting an acidosis.

A certain amount of evidence indicates that hemorrhaged dogs which died soon after transfusion were in a more serious condition immediately before volume replacement than were those in which transfusion produced permanent recovery. Thus in the terminal stage the former had a higher blood lactate, a greater arterial-jugular venous O_2 difference, a larger increase in the plasma amino nitrogen and a more severe acidosis than the animals in the latter group. The findings suggest that when the effects of hemorrhage attain a certain severity the restoration of the blood volume to near normal does not result in permanent recovery. This does not exclude the possibility that larger volumes might still be effective.

Comparison of whole blood and plasma transfusions. In our experiments the original blood volume was not restored completely by transfusion with either whole blood or with plasma. The deficit is partly explained by the volume of the blood samples taken for analyses. In the dogs transfused with plasma these amounted to 87 cc., whereas only 55 cc. were taken from the animals transfused with whole blood. The average time between hemorrhage and transfusion was $3\frac{1}{2}$ hours in the group transfused with plasma and $2\frac{3}{4}$ hours in the animals in which the volume was replaced

with whole blood. After transfusion the blood volume was smaller in the group which received plasma than in animals receiving whole blood. This may in part account for the lower post-transfusion blood pressure in the former group.

Transfusion with plasma lowered the hematocrit reading to almost half of the control value. As a result of such a large change in the red-cell-plasma ratio, the values for jugular venous O_2 content and arterial-jugular venous O_2 differences as well as the whole blood lactate concentrations found after plasma transfusions cannot be compared directly with those obtained following whole blood transfusion.

The decrease in the hematocrit readings produced by plasma transfusion results in a decrease in the relative viscosity of the blood (12). Other conditions remaining constant, a decrease in the relative viscosity reduces the resistance to flow. According to the work of Seligman *et al.* (13), the restoration of control blood volume with plasma may be expected to result in a greater blood flow than when the volume replacement is carried out with whole blood. If this is true, plasma transfused animals should show a smaller $A-VO_2$ difference than animals transfused with whole blood. Our data confirm this prediction. The average arterial-jugular venous O_2 difference measured one hour after plasma transfusion was 6 volumes per cent, whereas in dogs transfused with whole blood it was 10 volumes per cent. These values remained relatively constant for the succeeding 3 hours. If the O_2 consumption of the two groups of animals was of the same order of magnitude, the differences in the $A-VO_2$ values must indicate that the cardiac outputs of the plasma-treated dogs were higher than those of the animals which received whole blood.

SUMMARY

Thirty-seven dogs were bled according to a standard method and when the animals had reached the 'terminal stage' of hemorrhagic shock, whole blood or plasma was transfused in amount sufficient to return the blood volume to approximately the control values. In 4 animals volume replacement was carried out with saline. During the control period, immediately before transfusion and 4 hours after transfusion, the following determinations were made: plasma volume, hematocrit value, plasma protein concentration, arterial blood pH and O_2 and CO_2 content, whole blood lactate, plasma inorganic phosphate and amino nitrogen, urine volume and urinary phosphate concentration, jugular venous O_2 content, mean arterial blood pressure and heart rate.

The degree of recovery, as judged by the return to control values of the various measurements, was more complete in the dogs which were permanently resuscitated by transfusion than in the animals which died in spite of volume replacement. This is shown especially in the increased plasma bicarbonate concentration, in the decreased arterial-jugular venous O_2 difference and in the increased excretion of urine and urinary phosphate. The small extent and the transitory nature of the recovery after saline transfusion was striking.

The dogs which died after transfusion were in a more serious condition immediately before volume replacement, as judged by the magnitude of the changes in the blood constituents studied, than were those in which transfusion brought about recovery. This finding suggests that when the insult produced by diminished blood

volume is severe enough the return of blood volume to the control value does not result in permanent recovery.

Differences between the effects of whole blood and plasma transfusions appear to be related to the decrease in viscosity which occurs when plasma is used.

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STANDARDIZATION OF HEMORRHAGIC SHOCK IN THE DOG

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IN INSTITUTING a reliable experimental procedure for the production of shock, one must deal with the problem of selecting criteria for use in evaluating the degree of stress to which the animal is subjected (1). A review of the literature shows that many criteria have been used as guides in the production of hemorrhagic shock, and that the experimental animals have been hemorrhaged by somewhat different methods. Certain investigators (2, 3) have used a system of graded hemorrhages, the extent of which is based upon body weight. Others (4-9) have controlled the hemorrhage so as to maintain a chosen value of blood pressure for various periods of time. Frank, Seligman and Fine (10) employ extreme hypotension to produce shock. In this procedure the period of hypotension is terminated when one third of the shed blood is automatically taken up by the animal. Arimoto *et al.* (2) have used a system of graded hemorrhages in which blood pressure and arterial plasma CO₂ content taken after a chosen interval serve as a basis for calculating the survival time. Levine *et al.* (11) have produced irreversible shock in dogs by means of repeated bleedings which result in a depression of the arterial plasma CO₂ capacity to 15 volumes per cent or less when terminal conditions are reached.

In this laboratory a standard technique for the production of hemorrhagic shock was developed by Walcott (12). This procedure, which consisted essentially of a determination of the bleeding volume followed by reinfusion of a certain percentage of the shed blood, was used by Allison *et al.* (13, 14) in studying the chemical changes in blood, and the hemodynamic responses of dogs in hemorrhagic shock.

It is generally agreed that the chemical changes which occur in the blood of hemorrhaged animals result from anoxia, and the predominance of anaerobic metabolism in the tissues. On the basis of such findings Allison *et al.* (13) suggested that the extent of hemorrhage necessary to reduce the cardiac output to levels sufficient to cause shock might be gauged by certain chemical changes in the blood. They found that the most reliable indicator of the degree of shock in the dog was the concentration of plasma bicarbonate taken at the end of the period of reduced blood volume. The work of Allison *et al.* (13) formed a basis for the method to be described in this paper. In the present technique, not only the *magnitude* but also the *duration* of a depression in the plasma bicarbonate concentration is utilized as a guide in the control of hemorrhage and by this means shock of a determinable degree of severity is produced.

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METHODS

The animals used in this study were healthy mongrel dogs weighing between 7.4 and 15.9 kg. (av. 10.8 kg.). They were kept on a standard stock diet for a few days or until sufficiently fit for use. Each dog was examined on the day preceding the experiment and only those with leucocyte counts below 15,000/cu. mm., rectal temperatures below 39.5°C., and hematocrits above 35 per cent were used. On the same day, determinations of the blood and thiocyanate volumes were made by the method of Gregersen and Stewart (15). During the next 24 hours the animal was kept without food or water while urine was collected. The specific gravity of the urine samples of all the animals used in these experiments was greater than 1.020 indicating the absence of serious kidney damage.

On the following morning the dog was first offered water and was then transferred to the animal board. The animal was placed in a comfortable supine position, and a catheter inserted into the urinary bladder. Under local anesthesia (2 % novocaine subcutaneously, supported by neotheson) the right femoral artery was isolated for cannulation and branches of the left femoral artery and vein were exposed for sampling and infusion. During the course of the experiment each wound was kept covered with gauze moistened with saline.

Shortly after the control blood samples were obtained, the animal was bled from the right femoral artery according to the Walcott technique, and blood amounting to 25 per cent of the bleeding volume was reinfused via the bleeding tube. In the portion of blood which was immediately returned to the animal, clotting was prevented by the addition of a small amount of heparin solution. The remainder of the blood was allowed to run into a paraffined cylinder where it was mixed with 3.2 per cent sodium citrate. One volume of citrate solution was used for each 9 volumes of whole blood. The blood so collected was kept at room temperature until needed. After the reinfusion of blood, the cannula in the right femoral artery was connected to a mercury manometer for measurement of blood pressure.

During the course of the period of reduced blood volume the heart rate, respiratory rate and blood pressure were recorded before each blood sample was taken. From a branch of the femoral artery, 10 cc. or less of whole blood for various determinations was drawn anaerobically into syringes previously rinsed with heparin. Carbon dioxide and protein concentration of plasma, and *pH* and hematocrit values of whole blood were determined during the control period and at 30-minute intervals following the initial hemorrhage. The inorganic phosphate concentration of plasma, and glucose, lactate and pyruvate levels of whole blood were determined during the control period and 30 minutes and one hour following the initial hemorrhage. Thereafter these measurements were carried out at hourly intervals. The total volume of urine formed was determined several times during the period of reduced blood volume.

In certain experiments, at or between regular sampling times, the animal was subjected to several additional hemorrhages or transfusions of 10 to 50 cc. each. These adjustments of blood volume were employed in order to maintain the animal in the desired metabolic state. The extent of these adjustments was based mainly on plasma bicarbonate concentrations. To illustrate, during the 1 to 2 hours following the initial hemorrhage, additional small amounts of blood were withdrawn until

the plasma bicarbonate level had fallen to 6 mm/l or less. Subsequently, small hemorrhages or transfusions were employed in order to maintain the plasma bicarbonate concentration at or slightly below this level. Experiments in which the period of reduced blood volume was shorter than 2 hours were discarded.

At the completion of the period of reduced blood volume, the manometer was removed and the blood which had been collected in the paraffined cylinder was filtered through gauze and injected via the femoral artery over a period of 2 to 4 minutes. The first portions were given rapidly and the remainder more slowly. On the average, the blood lost in filtering and sampling amounted to 12 per cent of the control blood volume. Use of blood from a donor animal to make up this deficiency was avoided because of the possibility of complications arising from incompatibility of bloods from different sources.

Immediately after the transfusion, the manometer was reconnected, and all the determinations previously mentioned were continued during the next 4 hours. Results obtained during this post-transfusion period will be found in another paper (16). At the end of this 4-hour period, the vessels were tied off, the wounds washed with saline, dusted with sulfathiazole and closed with widely separated sutures to allow drainage. No subsequent infections were noted. The animal was then returned to a cage and kept 12 hours without water. Dogs alive at the end of this period were considered to be survivors.

The determinations of bicarbonate, inorganic phosphate and protein concentration of plasma as well as lactate and pyruvate levels and pH of whole blood were carried out as previously described by Root *et al.* (17). Whole blood glucose was estimated according to the technique of Somogyi (18, 19). Hematocrit values were measured with Wintrobe tubes.

RESULTS

Certain of the physiological responses which occur following severe hemorrhage are illustrated in figures 1 and 2. Such data, obtained on dogs subjected to a period of reduced blood volume which was followed by transfusion, were examined for criteria which could be used to separate survivors from non-survivors. The best results were obtained by taking into account the extent and duration of the depression of plasma bicarbonate content. The assumption was made that the product of an intensity factor and time would give a measure of the total amount of injury sustained by the animal. Accordingly, plots of plasma bicarbonate concentration vs. time were made for the period of reduced blood volume. The area enclosed by such a plot and a line drawn at plasma $BHCO_3$ 10 mm/l was used as a measure of total injury and was called 'stress index' (S.I.). In the plots prepared from the data of these experiments, the stress indices represent an area measured in terms of millimole minutes.

The data in table 1 show the relationship between stress index and mortality of shocked animals treated with whole blood transfusion or with whole blood transfusion plus supplement. The latter group is included in the table for purposes of comparison; however both of these groups of dogs may be considered to be alike since it has been shown (16) that the supplementary treatment does not influence mortality. The animals with S.I. below 420 were considered to be lightly injured and those with S.I. above 420 were considered to be severely injured.

Figures 1 and 2 illustrate average results obtained on a group of 16 animals with S.I. less than 420 and 17 animals with S.I. greater than 420. In order to obtain curves for each group, the period of reduced blood volume of each animal was divided

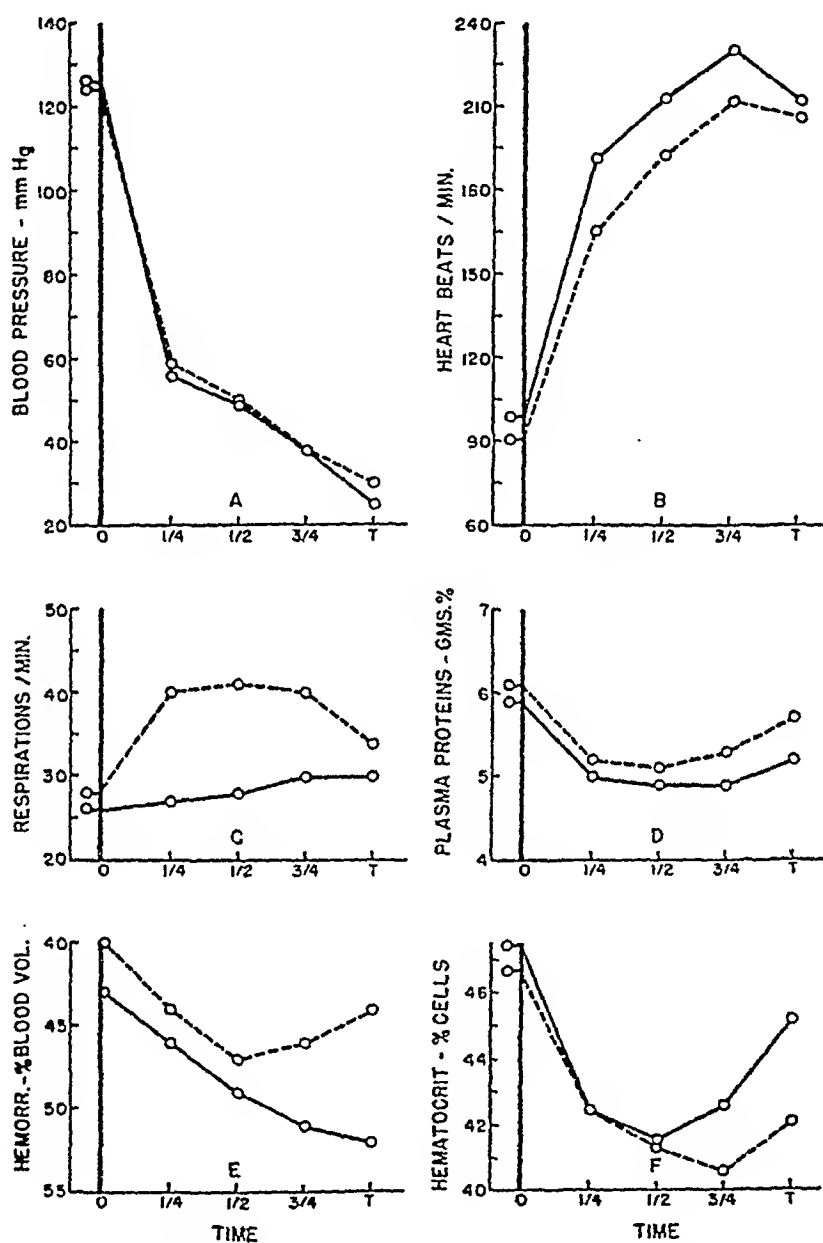


Fig. 1. SHOWING THE EFFECT OF STANDARD HEMORRHAGE on the blood pressure, heart rate, respiratory rate, plasma proteins and hematocrit. The initial bleeding is represented by the vertical bar at zero time, and the period of reduced blood volume which follows is divided into four quarters. The total amount of blood withdrawn during the initial bleeding and during the period following is expressed as a percentage of the control blood volume. Solid lines indicate a series of 16 dogs with stress index less than 420; broken lines, a series of 17 dogs with stress index greater than 420.

into quarters and data obtained at the end of similar quarters of each experiment were averaged. When heavily and lightly damaged animals are so compared it is found that such differences as occur appear to be quantitative, not qualitative. The curves

indicate that animals with the greater amount of injury as judged by stress indices, when compared with those of lesser injury, withstand less blood loss, respire more rapidly and have less cardiac acceleration. The hematocrit values of both groups

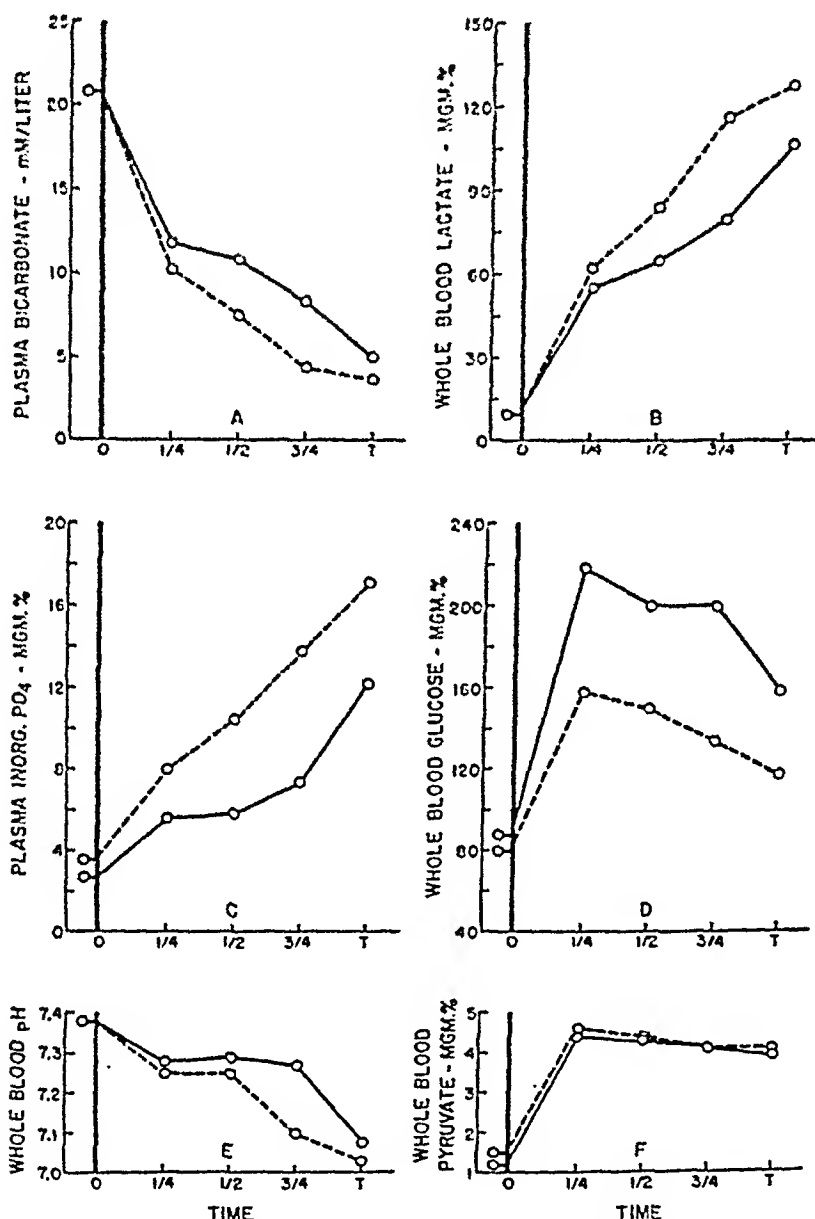


Fig. 2. SHOWING THE EFFECT OF STANDARD HEMORRHAGE on the plasma bicarbonate and phosphate, and on the whole blood glucose, lactate, pyruvate and pH. The initial bleeding is represented by the vertical bar at zero time and period of reduced blood volume which follows is divided into four quarters. Solid lines indicate a series of 16 dogs with stress index less than 420; broken lines, a series of 17 dogs with stress index greater than 420.

are equally depressed for the first half of the period of reduced blood volume, but for the second half of this period a greater rise is shown by the lightly damaged animals. There is no significant difference between the blood pressure, plasma protein concentration or whole blood pyruvate of each series. Further, the more heavily damaged dogs show less hyperglycemia, lower pH, higher whole blood lactate and

higher plasma inorganic phosphate than the lightly damaged animals. A comparison of the plasma BHCO_3 curves shows that the terminal values of this constituent are not greatly different in the two series. However, it is important to note that in the severely damaged group the plasma BHCO_3 content is depressed below 10 mm/l for an average time of 131 minutes as compared with an average time of 105 minutes for the lightly damaged group. Putting it differently, it may be said that the severely damaged animals have been subjected to a longer period of intense stress than that of the lightly damaged animals.

The average length of the period of reduced blood volume was 240 minutes for dogs with S.I. less than 420, and 198 minutes for the dogs with S.I. greater than 420.

No regular relation could be found between the available fluid volume expressed as cc/kg. body weight, and the degree of plasma dilution after the initial hemorrhage as determined by changes in the plasma protein concentration. Practically no urine was formed during the period of reduced blood volume.

TABLE 1. RELATION BETWEEN THE SEVERITY OF SHOCK, AS DETERMINED BY STRESS INDEX, AND MORTALITY IN DOGS SUBJECTED TO A PERIOD OF REDUCED BLOOD VOLUME FOLLOWED BY TREATMENT WITH WHOLE BLOOD TRANSFUSION OR WHOLE BLOOD TRANSFUSION PLUS SUPPLEMENT

Stress Index.....	SURVIVORS		NON-SURVIVORS	
	Below 420	Above 420	Below 420	Above 420
Whole blood transfusion	5	2	1	4
Whole blood transfusion, plus glucose or NaHCO_3	8	3	2	8

The average bleeding volume of 52 dogs was found to be 54.7 cc/kg. body weight. Total blood volume determinations on these animals indicates that the bleeding volume represents 55.4 per cent of the total blood volume. These data are in substantial agreement with those obtained more recently on a large series of dogs by Walcott (20).

DISCUSSION

In the study of the physiological changes which occur in hemorrhagic shock, and in the evaluation of therapeutic measures used in its treatment, it is essential to have some gauge of the degree of stress to which the animal is subjected. Much of the earlier work of this laboratory was done with this idea in mind. Allison *et al.* (13) conducted a series of experiments on dogs which were hemorrhaged according to the Walcott technique (12). In these experiments, the animals were given whole blood transfusion when the blood pressure had reached 25 mm. Hg or less and was falling rapidly. The results showed that with non-survivors the plasma bicarbonate content at terminal conditions was less than 6 mm/l while for survivors it was above this value.

Our early attempts to repeat the work of Allison were not entirely successful in one respect. We found that a number of animals with terminal plasma bicarbonate content less than 6 mm/l recovered when treated with whole blood transfusion.

Apparently the dogs of Allison's series were more susceptible to the effects of hemorrhage than those used in the present study.

In order to raise the mortality in our experimental animals, a different line of approach was taken. It seemed reasonable in determining the severity of shock to take into account not only the intensity of the shock-producing process, but also the length of time during which the process acts. Gregersen has referred to this concept in a recent paper on shock (21). Proceeding with this idea in mind, the magnitude of the reduction of plasma bicarbonate content occurring during a period of reduced blood volume was used as a measure of the intensity of the shock-producing process. The product of this intensity factor and time was utilized as a means of evaluating the degree of stress to which the animals were subjected. The stress indices so determined were found to be more reliable than any other physiological criterion when employed for predicting the mortality of a group of dogs treated with whole blood transfusion in the manner previously described.

It may appear unusual to gauge the severity of shock by means of an area measured in millimole minutes but it is interesting to note that a somewhat similar idea may be found in the work of Wiggers and Werle (8). These investigators showed that in dogs subjected to hemorrhage, both the magnitude of the reduction of blood pressure as well as the duration of the period of hypotension were important in determining the severity of shock. However, they did not extend the work to permit quantitative relationship to be set up between these factors.

By use of the method outlined here one may reproduce experimentally a group of animals of nearly equal stress indices and demonstrate that the mortality rate is related to the degree of shock imposed. Such an arrangement is made possible by the fact that the stress index may be periodically determined during the period of reduced blood volume, and when the desired degree of shock is attained, the therapeutic procedure may be instituted. It is well to bear in mind, that only if the stress index lies at the extremes of the scale may one predict with a high degree of certainty the survival or death of any particular animal following hemorrhage and transfusion. For example, a group of animals with high stress indices might be prepared. Such a group would probably show high mortality rates if treated only with whole blood transfusion, and according to the current definitions these animals could be considered to be in 'irreversible' shock. If one wishes to study the effectiveness of various therapeutic measures in the treatment of severe shock it is possible by this method to prepare such a group of 'irreversibly' shocked animals.

The limitations of data of these experiments do not permit a rigid statement of the relationship between S.I. and the percentage survival following transfusion (table 1). From the data available it would seem that under the conditions of these experiments, a group of animals having stress indices between 340 and 480 should have a survival rate of about 50 per cent when treated with whole blood transfusion.

The physiological changes in hemorrhagic shock as reported in this paper have been repeatedly observed (1, 13, 14, 17). It is rather interesting to note in comparing the average data on the series of dogs with stress indices greater than 420 with those having stress indices less than 420, that the blood pressure curves are identical for the two groups (fig. 1A). This result lends support to the statement that blood pressure alone is an unreliable criterion of the severity of shock (1). The data in this paper support the idea that not only the intensity of tissue anoxia but also its duration are factors which determine the extent to which an animal is damaged by hemorrhage.

A point worth noting (fig. 1E) is that the animals with stress indices greater than 420 have smaller reduction in their circulating blood volume than the animals with

stress indices less than 420. The first group also show less cardiac acceleration (fig. 1B) than the second which may indicate that the former group has inherently a poorer circulatory compensatory mechanism.

SUMMARY

A method of producing hemorrhagic shock in dogs is outlined in which the severity of shock is gauged by means of stress indices. In this method, stress indices are calculated by taking into account the extent and duration of a depression of plasma bicarbonate content which occurs during a period of reduced blood volume. The mortality rate in a series of 6 dogs with stress indices in the range of 0 to 420 is 17 per cent while that in an equal number of dogs having stress indices ranging from 420 to 840 is 67 per cent.

Comparison of the average results obtained on a series of 17 dogs having stress indices between 420 to 840 (severe stress) with a second series of 16 dogs having stress indices between 0 to 420 (moderate stress) shows that during the periods of reduced blood volume the animals of the former group withstand less blood loss, respire more rapidly, have less cardiac acceleration and show less hyperglycemia. Further, dogs subjected to severe stress show greater increases in whole blood lactate and plasma inorganic phosphate, and a greater depression in plasma bicarbonate and whole blood pH than those subjected to moderate stress. Both groups of animals show an early fall in hematocrit followed by a later rise, this rise being more pronounced in the series with stress index between 0 to 420. The reduction in blood pressure and in plasma protein concentration and the elevation in whole blood pyruvate are not significantly different in both groups.

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THERAPY IN HEMORRHAGIC SHOCK: EFFECTS OF SUPPLEMENTATION OF WHOLE BLOOD TRANSFUSION WITH GLUCOSE AND WITH SODIUM BICARBONATE

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THE efficacy of whole blood transfusion in the treatment of hemorrhagic shock is well recognized. However, in severe, sometimes called irreversible shock, restoration of the blood volume to original levels may not be sufficient to maintain life (1, 2). In such instances the question arises as to the value of treating shock with whole blood transfusions to which supplementary agents have been added. Furthermore, in cases where treatment with whole blood transfusion alone brings about recovery, it would be of interest to determine whether supplementary agents speed the recovery process.

Recent reports as to the value of supplementation of transfusions in the treatment of hemorrhagic shock are not in agreement. Wiggers and Ingraham (3) state that alkalinizing agents are of value in preventing the transition from impending to irreversible hemorrhagic shock. Levine *et al.* (4) have shown that both NaHCO_3 and glucose when used as adjuncts to whole blood transfusion decrease the mortality rate in 'irreversible' hemorrhagic shock. Frank *et al.* (1) conclude that NaHCO_3 has no efficacy when used under such conditions. The uncertainty as to the value of supplementation of whole blood in the treatment of hemorrhagic shock made it appear worth while to reinvestigate the problem.

It has been clearly shown that certain marked physiological changes from the control condition occur when an animal is subjected to hemorrhage (5-8). During the recovery process those elements which have been shifted gradually return to their original values. As Allison *et al.* (6) have pointed out, the rate and completeness of this return seem to depend to a large extent on the degree of injury sustained by the animal during the period of stress, as well as upon the effectiveness of the therapy given.

In another paper an attempt to develop a procedure for gauging the intensity of shock produced by hemorrhage has been outlined (8). It seemed likely that by use of this method and by analysis of certain physiological and biochemical changes occurring during recovery from hemorrhagic shock, that an accurate estimate of the effectiveness of various therapeutic measures might be made. The experiments reported here are concerned with three groups of dogs which have been subjected to hemorrhagic shock of comparable degree of severity, as measured by stress indices (8). One series has been treated with whole blood transfusion, the second with whole blood transfusion plus sodium bicarbonate, and the third with whole blood transfusion plus glucose. The mortality and rates of recovery of the three groups have been compared.

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METHODS AND RESULTS

The methods used have been outlined in another paper (8). At the close of the period of reduced blood volume the animal was transfused, and all the determinations were carried out at the elapsed times of 30 minutes, 1, 2, 3, and 4 hours. On the average, in each of the three series of dogs, the blood lost in filtering and sampling amounted to 12 per cent of the control blood volume. Supplemental therapy was introduced between the 30 minute- and 1-hour samples. Ten per cent NaHCO_3 or 30 per cent glucose were given at the rate of 1 or 2 cc/min. via a branch of the femoral vein. The dosages were NaHCO_3 -0.5 gm/kg., glucose-0.54 gm/kg. Hypertonic solutions were used in order to minimize the volume of the material injected. Twelve dogs were treated with whole blood transfusion, ten with whole blood transfusion plus NaHCO_3 and eleven with whole blood transfusion plus glucose.

The number of survivors and non-survivors on three types of therapy is given in table 1. It can be seen that the stress indices (S. I.) of the three groups of survivors as well as the stress indices of the three groups of non-survivors are fairly comparable.

TABLE 1. COMPARISON OF THE MORTALITY OF THREE GROUPS OF SHOCKED DOGS GIVEN THREE TYPES OF THERAPY

THERAPY	SURVIVORS		NON-SURVIVORS	
	No. Animals	Av. S.I.	No. Animals	Av. S.I.
Whole blood trans.....	4	391	5	468
Whole blood trans. + NaHCO_3	4	393	5	502
Whole blood trans. + glucose	7	370	4	496

The physiological responses representing average results on three groups of animals following transfusion, or transfusion plus supplemental therapy, are given in figures 1 to 3. The data show that as compared with that of survivors, the rate and extent of recovery are less in the non-surviving animals, especially in those dying before the end of the 4-hour period of study.

The infusion of NaHCO_3 raised the level of plasma bicarbonate to control values, and it can be seen (fig. 3B) that only the survivors maintained this concentration. Further, these dogs showed increases in whole blood pH (fig. 2H), and increases in pyruvate (fig. 2E) and lactate concentrations (fig. 2B).

The infusion of glucose raised the blood level of this substance well above control values (fig. 3F). In the survivors, the blood glucose concentration was reduced to 80 mg. per cent (original control values) in the 2 hours immediately following the completion of the infusion, while the same reduction required about 3 hours in the non-survivors. According to Mann (9) the nonanesthetized normal dog given an equal dose of glucose will restore the increased blood levels to control values in 30 minutes to one hour.

The total volume of urine formed by these three groups of animals during the 4-hour post-transfusion period is given in table 2. For all cases the urinary output is greater in survivors than in non-survivors.

In order to compare the rate of disappearance of lactate in hemorrhaged-

transfused animals with that of control animals, 2 dogs were injected with sodium-D-lactate (1 gm/kg. body weight) and the blood level of this constituent was followed for 4 hours. In addition, determinations of whole blood pyruvate and pH , and

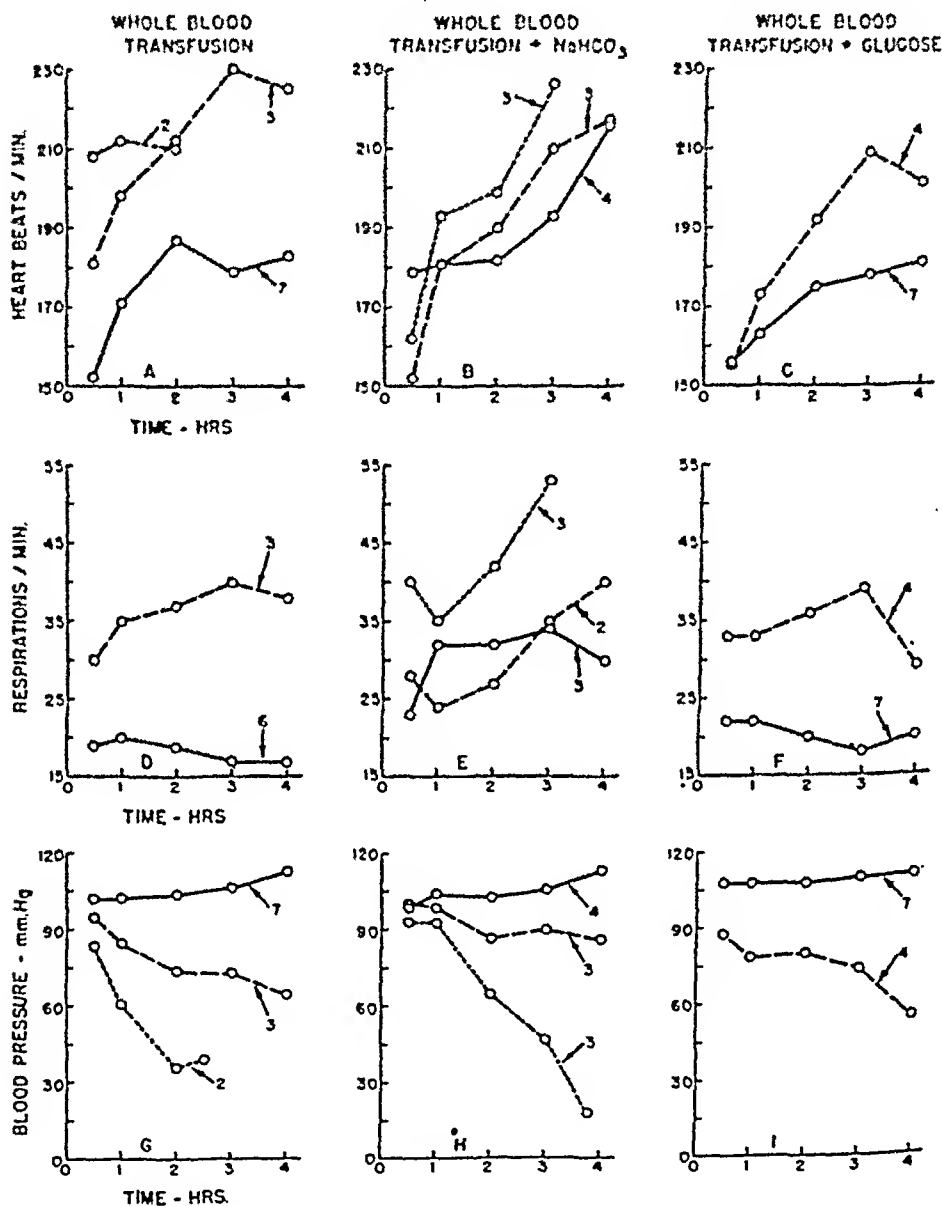


Fig. 1. SHOWING THE CHANGES in the heart rate, respiration and blood pressure in survivors and in non-survivors after an infusion of whole blood, whole blood plus bicarbonate, and whole blood plus glucose. Short dashes indicate non-survivors dying in less than 4 hours, long dashes indicate non-survivors dying in more than 4 hours, solid lines indicate survivors. The number of animals in a group is indicated by the numeral associated with each curve.

plasma $BHCO_3$ and PO_4 were carried out. The results are shown in figure 4. It can be seen that the concentration of lactate in whole blood reaches 105 mg. per cent, a value which is about equal to that of hemorrhagically shocked dogs shortly after therapeutic treatment with whole blood transfusion. The results show that the rate

of disappearance of lactate from the blood of control dogs is of the same order as that of shocked animals during the post-transfusion period (compare fig. 4 with fig. 2A). It is also interesting to note that in the sodium-D-lactate injected animals the lactate/-

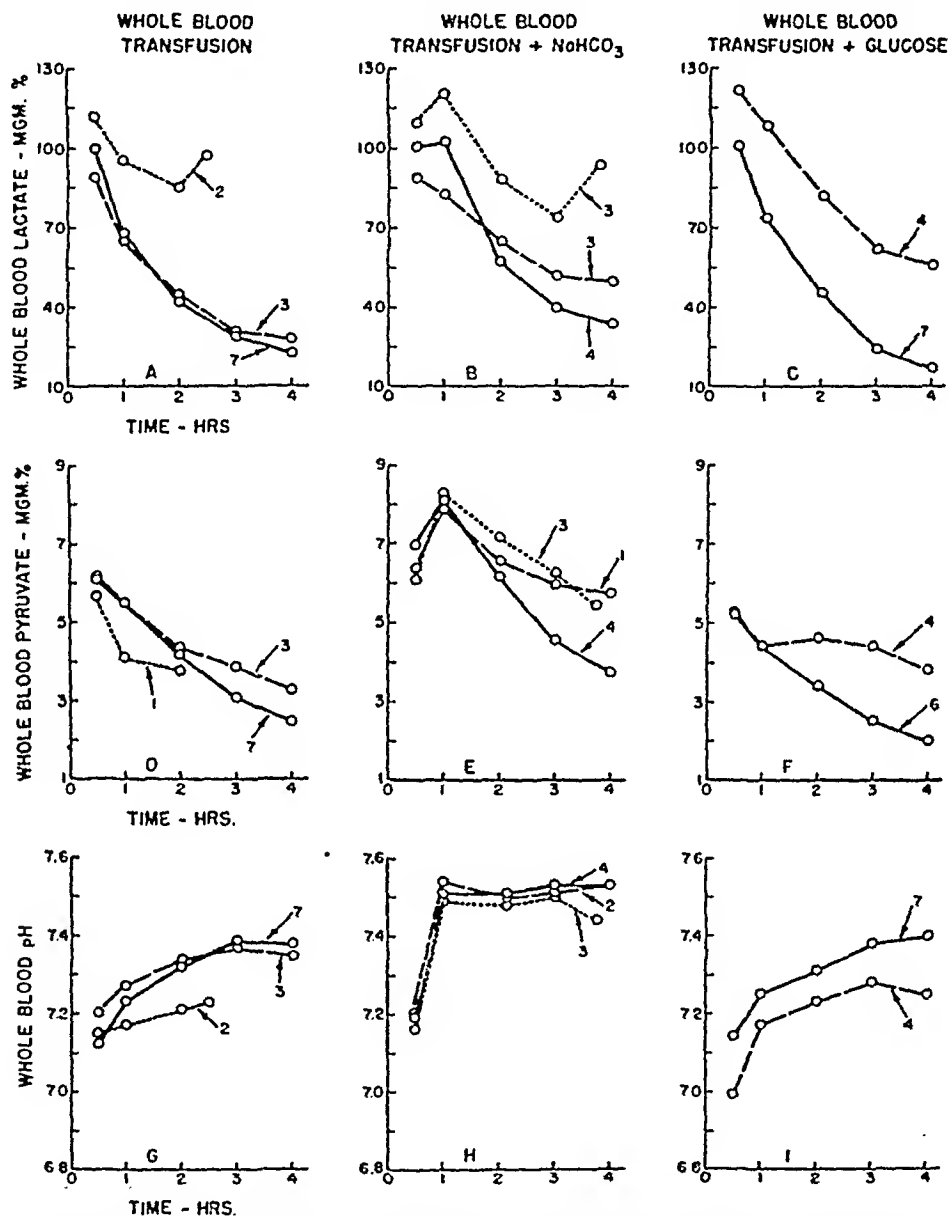


Fig. 2. SHOWING THE CHANGES in whole blood lactate, whole blood pyruvate and whole blood pH in survivors and in non-survivors after an infusion of whole blood, whole blood plus bicarbonate, and whole blood plus glucose. Short dashes indicate non-survivors dying in less than 4 hours, long dashes indicate non-survivors dying in more than 4 hours, solid lines indicate survivors. The number of animals in a group is indicated by the numeral associated with each curve.

pyruvate ratio remained close to the control value during the entire 4-hour period. In the case of the shocked animals, the lactate/pyruvate ratio was considerably higher. In addition, dogs given sodium lactate formed plasma bicarbonate at a more rapid rate than the shocked-transfused animals.

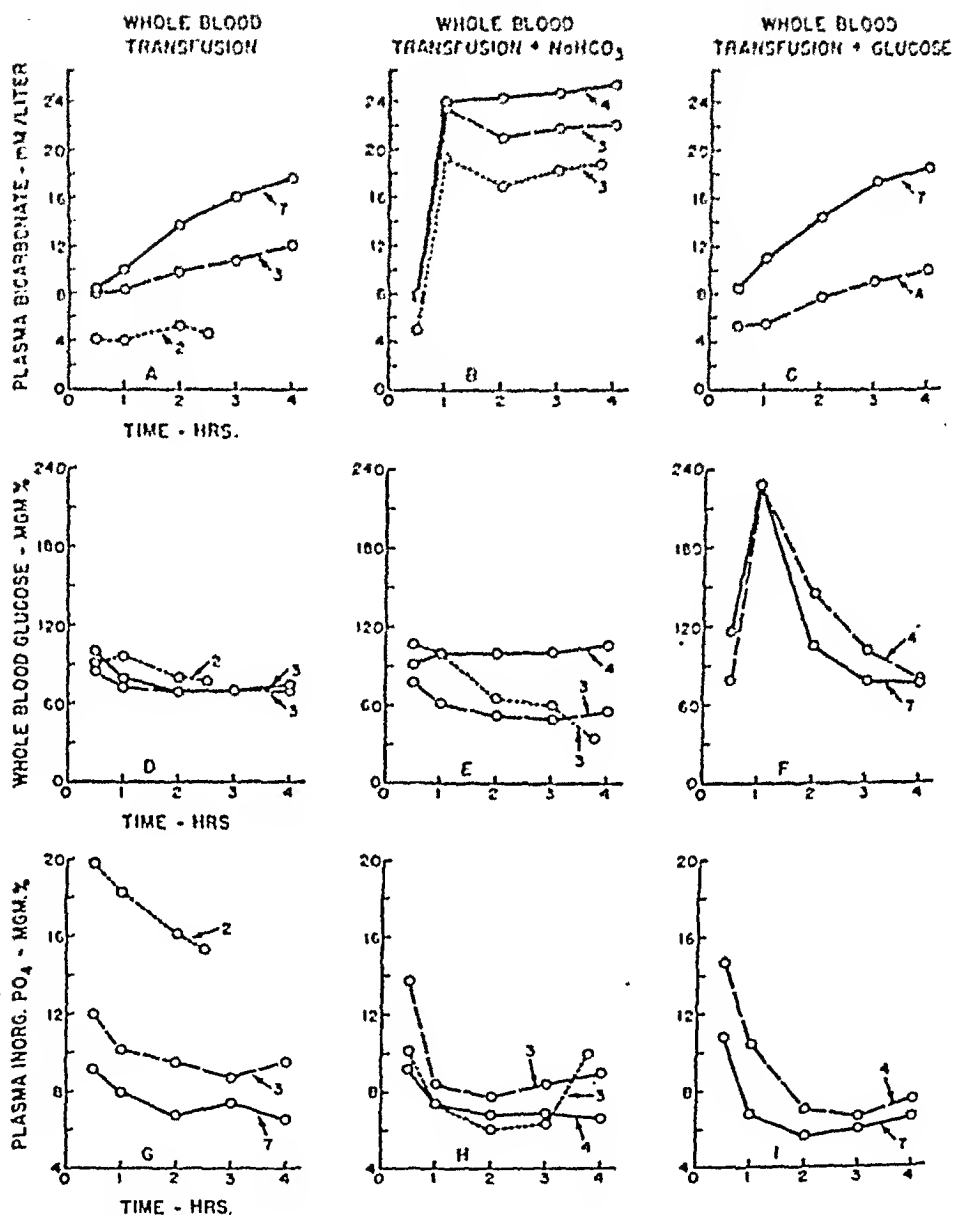


Fig. 3. SHOWING THE CHANGES in plasma bicarbonate, whole blood glucose and plasma inorganic phosphate in survivors and in non-survivors after an infusion of whole blood, whole blood plus bicarbonate and whole blood plus glucose. Short dashes indicate non-survivors dying in less than 4 hours, long dashes indicate non-survivors dying in more than 4 hours, solid lines indicate survivors. The number of animals in a group is indicated by the numeral associated with each curve.

TABLE 2. TOTAL VOLUME OF URINE FORMED BY SHOCKED DOGS DURING A 3 1/2-HOUR PERIOD FOLLOWING THREE TYPES OF THERAPY

THERAPY	SURVIVORS		NON-SURVIVORS	
	No. Animals	Urine Vol., cc.	No. Animals	Urine Vol., cc.
Whole blood trans.....	7	73	5	25
Whole blood trans. + NaHCO ₃	3	170	5	37
Whole blood trans. + glucose	6	115	3	34

The blood pressure, respiratory rate and heart rate of hemorrhaged animals given whole blood transfusion at first tend to move toward control values and later show changes similar to those which occur following the initial shock producing hemorrhage.

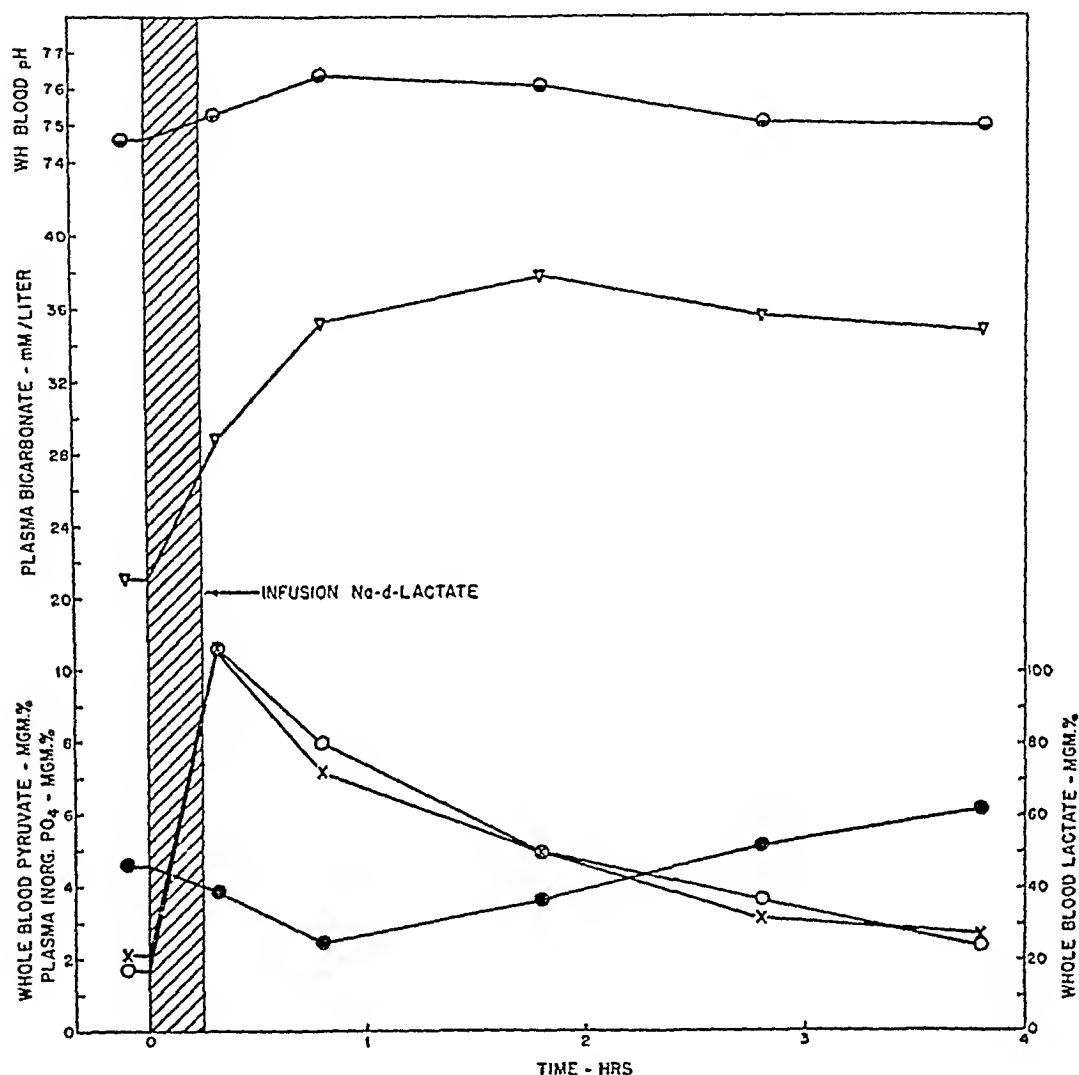


Fig. 4. SHOWING THE EFFECT of infusion of sodium-D-lactate (1 gm/kg. body weight) on pH and on the concentrations of lactate and pyruvate of whole blood as well as upon the concentrations of inorganic phosphate and bicarbonate of plasma.

Open circles = lactate Closed circles = PO_4 Crosses = pyruvate
 Half-closed circles = pH Triangles = $BHCO_3$

No striking differences were seen in the plasma protein concentrations or hematocrits of the three groups of dogs.

DISCUSSION

The physiological responses of hemorrhagically shocked dogs treated with whole blood transfusion indicate that those with the greatest degree of damage as judged by stress indices show greatest mortality, and a relatively sluggish incomplete recovery from the condition in which they are placed as a result of hemorrhage.

The data of table 1 show that supplementation of whole blood transfusion to the extent previously indicated (see METHODS AND RESULTS) has no striking effect on the mortality of animals subjected to equivalent degrees of damage. In preparing this table it was found that the average stress index values of the three groups of survivors and of the three groups of non-survivors could be made more nearly comparable if results on certain animals were not included. For this reason 3 survivors (treated with whole blood transfusion) which had low stress indices, and one non-survivor (treated with whole blood transfusion plus sodium bicarbonate) which had a very high stress index do not appear in the final tabulation.

Survey of the data given in figures 1 to 3 shows that the supplemental therapy does not appear to influence greatly the recovery process of either lightly or heavily damaged animals. In the case of NaHCO_3 -treated animals, it is interesting to note that although this substance raised the plasma BHCO_3 level to control values, these heightened concentrations were maintained only by the survivors. The rate at which lactate and pyruvate are removed from the blood was low in all non-survivors and this condition is not improved by either type of supplemental therapy.

Examination of the blood glucose curves of animals given glucose infusion shows that the non-survivors are successful in removing this substance from the blood, although at a lower rate than that of the survivors. During the 4-hour post-transfusion period the non-survivors excreted the equivalent of 2 per cent of the injected glucose in the urine, and at the same time they formed 4 mEq. of bicarbonate/liter of plasma. If this rate of bicarbonate formation in plasma is paralleled by equal rates in the total body fluids, and if it is assumed that the injected glucose is the sole source of the newly formed bicarbonate, then one can only account for about one-third of the infused glucose. In the light of Haist and Hamilton's observations (10) on the failure of rats shocked by tourniquet application to store injected glucose, it would be interesting to determine whether glycogen storage occurs under the conditions of these experiments. It may be noted that for certain of the charts of figures 1 to 3, the number of animals included is less than the total number of dogs per series. It was necessary to discard some of the pyruvate determinations because of analytical difficulties. Further, analyses of whole blood glucose were not carried out in the early experiments and therefore these data are not available for 4 of the survivors which had been treated with whole blood transfusion. In figure 1D, respiratory rates for the 2 non-survivors which died in less than 4 hours were omitted because these animals panted throughout most of the experimental period.

As has been previously stated, in all of these experiments about 12 per cent of the control blood volume was consumed in handling and sampling. Although no actual measurements were made, it is not unlikely that in the period following whole blood transfusion, the circulating blood volume of these animals was less than the control values. Considering the efficacy of whole blood in the treatment of shock, it would be of interest to investigate the effects of increasing the volume of blood given in transfusion and to compare the results with those of the present series. Improvement in the rate of recovery and mortality might result when the blood volume is raised to 125 or 150 per cent of the control value.

SUMMARY

The effectiveness of whole blood transfusion and whole blood transfusion plus NaHCO_3 or glucose in the treatment of hemorrhagic shock has been compared. Therapeutic value was judged on the basis of mortality and also on rates of recovery from shock. The results show that the two supplemental agents when used under the conditions of these experiments cannot be considered to be of great effectiveness as therapeutic adjuncts to whole blood transfusion in the treatment of shock.

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NORMAL BLOOD VOLUME, PLASMA VOLUME AND THIO-CYANATE SPACE IN RATS AND THEIR RELATION TO BODY WEIGHT¹

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ALTHOUGH there have been several reports of the normal blood and plasma volumes in rats, the most complete to date have been those of Metcoff and Favour (1) and of Lippman (2). The former authors used the familiar Evans Blue dye dilution method while Lippman used the dilution of an injected hemoglobin solution. Previous values from the literature were tabulated in both of these papers for comparison with the values obtained. The absolute values reported by Metcoff and Favour and by Lippman are in reasonable agreement, but the conclusions drawn of the relation of these fluid volumes to body size differ considerably. Lippman plotted the data as logarithms and calculated the regression line obtained. Both blood and plasma volume were found to vary in proportion to approximately the same power of body weight, $Wt^{.794}$ for blood volume, $Wt^{.773}$ for plasma volume. An inspection of the scatter diagram presented shows, however, that the data do not fit the regression line very well for small animals. Those weighing less than 100 grams fell below while those that weighed about 100 grams fell generally above the line. The author suggests that changes in the amount of body fat, a relatively avascular tissue, might be the cause of this poor fit but if such were the case one might expect the fit to be poorest for the largest animals rather than for the smallest ones.

Metcoff and Favour, on the other hand, concluded that while plasma volume was not constant in relation to either surface area or body weight in young rats weighing less than 100 grams, the plasma volume of animals above this weight was reasonably constant per unit of surface area, about 2.90 cc per 100 cm.². Since surface area was calculated from Lee's formula (3) which describes surface area in cm.² as equal to $12.54 Wt^{.6}$, if plasma volume varies directly with surface area it must vary directly with $Wt^{.6}$ as well. This is much different from the value of $Wt^{.8}$ obtained by Lippman.

Whereas Lippman found both blood and plasma volume to vary with about the same power of body weight, Metcoff and Favour reported no constant relation of blood volume with body weight or surface area. In spite of this finding, Metcoff *et al.* (4) in a later report have calculated blood volumes per unit of surface area also.

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In a previous paper (5) we have described the methods developed for the determination of plasma and blood volumes and thiocyanate space in rats. The methods were modifications of those already familiar and Evans Blue was used in the determination of the blood and plasma volumes. Normal values were obtained using 69 rats weighing from 63 to 394 grams. Since the values obtained were similar to those reported by Metcoff and Favour, who used a similar method, we have combined their data with our own to increase the number of determinations and facilitate the analysis of the relation of these fluid volumes to body size.

Thiocyanate space has apparently not been previously determined in rats. Its relation to body size as well as to the blood and plasma volumes has also been studied.

EXPERIMENTAL

The data were obtained as previously described (5). The animals were supposedly normal, from a strain originally derived from the Wistar strain, and they received a diet of Purina dog chow and water. Food and water were present in the

TABLE 1. DESCRIPTION OF ANIMALS FROM WHICH DATA WERE OBTAINED

AUTHOR	GROUP	SEX	WEIGHT RANGE	NUMBER OF ANIMALS		
				Blood vol.	Plasma vol.	Thiocyanate space
Metcoff & Favour	1	M	gm. 41-68	12	12	
	2	M	73-89	9	9	
	3	M	137-336	13	13	
Wang & Hegsted	4	F	63-80	10	10	9
	5	F	114-136	10	10	10
	6	F	168-294	42	42	26
	7	M	281-394	7	7	7

cages until the time the determinations were done. Metcoff and Favour (1) used Sherman strain animals which were fed a purified diet. Their animals were fasted for 4 hours prior to study.

The data thus available are indicated in table 1. The animals have been grouped according to certain weight limits, and the groups numbered to facilitate the discussion. The method used in the analysis has been that already extensively used in studies upon relative growth by Huxley (6) and more recently by Brody (7) and others. These and other authors have shown that the relation of organ weight as well as metabolic rates, food intake, etc. may be well represented by equations of the type: $Y = a Wt^b$.

It may be helpful to point out that the exponent b is the relative increase in Y with respect to Wt , or if the decimal be moved two places to the right, it is the percentage increase in Y when Wt increases 100 per cent or is doubled. Thus if a value of 1 is obtained for b , Y and Wt increase proportionally and in this event a straight line relationship would be obtained in an arithmetic plot.

The equation is obtained as described by Brody (7) by plotting the data as logarithms and calculating the regression line which has the form: $\log Y = b \log Wt + \log a$. Removal of the logarithms results in the familiar equation. However, it should also be mentioned that two regression lines may be calculated for each group of data. The value of b may thus be either b_y or b_x depending upon the line calculated. The line with the deviations measured in the Y direction which gives b_y is the one ordinarily calculated. Zucker (8) points out that the value of b_y is always smaller than b_x , and that the true line is expected to fall between the two calculated lines. The value found for b_y must therefore be a near minimum estimate. Regression lines were calculated for the data indicated in the figures which follow, through different weight ranges as well as for the combined data. The determination of the correlation coefficients, standard errors of estimate, and the standard error of the slope, b_y , were used as well as logic to arrive at the lines representing the best fit of the data. Since increasing the range upon the x axis will ordinarily improve the correlation coefficient, this must be borne in mind in interpreting the results.

Finally, it may be expected from the data of Huxley (6), Brody (7), and others summarized by Brody, that one line may not accurately depict the values over the entire weight range of the animals. Growth rate, metabolic rate, liver weight, etc. all show a marked change in their relation to body weight in rats weighing approximately 100 grams, which is approximately at the time of puberty. Our findings indicate this to be true for blood and plasma volumes and thiocyanate space as well.

RESULTS AND DISCUSSION

Figure 1 is a scatter diagram showing values obtained for the blood volume of rats of various sizes. Although the reader's impression of the data is influenced by the lines which have been drawn, it seems apparent that two lines are required to adequately represent the data. The break is clearly between 100 and 150 grams of body weight but whether the data falling within this region should be included with the smaller animals, the larger animals, or excluded from either line might be debatable.

The over-all line has the equation $BV = .191 Wt^{.821}$. This is essentially the same as that obtained by Lippman. Table 2 shows the statistics calculated for the regression lines over the various weight ranges. Inclusion of the group weighing between 114 and 136 (*group 5*, table 1) with the smaller animals improves the correlation, whereas inclusion with the larger animals markedly decreases the correlation. From this analysis there would appear to be no logical reason for not including all of the animals up to 140 grams in weight as one group, and the animals above this weight as another more or less homogeneous group. The value of the exponent b for both of these lines is approximately 1. Therefore it appears that blood volume varies approximately as the body weight in these two weight ranges, 40 to 140 gm., 140 to 400 gm. When the body weight is doubled the blood volume is also approximately doubled and blood volume may logically be expressed as percentage of body weight. In the smaller animals it is approximately 9 per cent of the body weight, while in adult animals it is approximately 7 per cent. In the transition zone any estimate will clearly be of dubious value.

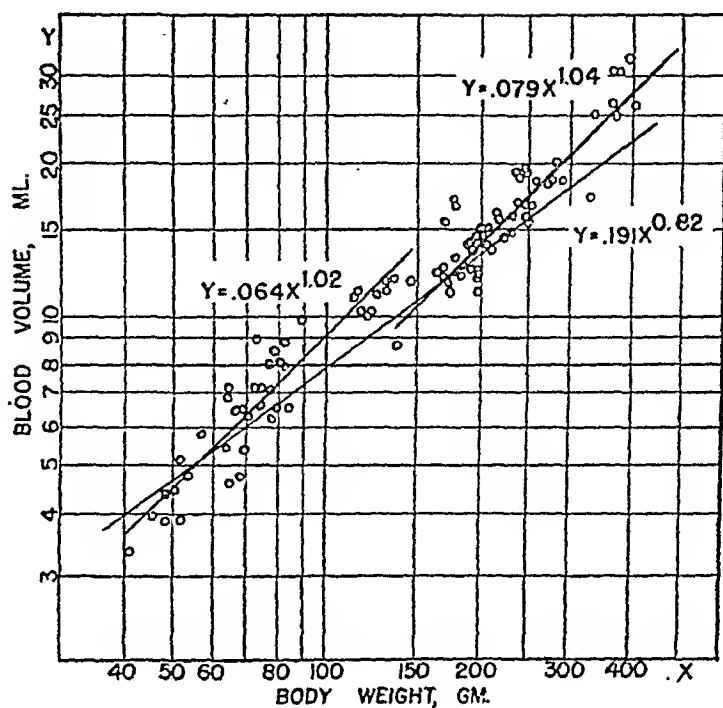


FIG. 1. RELATION of blood volume to body weight in rats.

TABLE 2. EFFECT OF RANGE IN WEIGHT UPON VARIOUS REGRESSION LINES CALCULATED AND THE RELIABILITY OF REGRESSION LINES

COMPARISON MADE	WEIGHT RANGE	NO. OF ANIMALS	'a'	b ± S _b	r
	gm.				
Blood vol. vs. wt.	40-100	31	.0417	1.19 ± .117	.88
	40-136	41	.0785	1.04 ± .061	.94
	120-400	72	.0049	0.49 ± .094	.42
	137-400	62	.064	1.02 ± .068	.89
	40-400	103	.192	0.82 ± .023	.96
Plasma vol. vs. wt.	41-100	31	.027	1.169 ± .172	.86
	41-136	41	.073	.932 ± .069	.91
	114-400	72	.175	.725 ± .041	.90
	136-400	62	.118	.800 ± .071	.82
	41-400	103	.182	.723 ± .022	.96
Hematocrit vs. wt.	41-400	103	20.37	.146 ± .016	.67
Thio. sp. vs. wt.	63-136	19	.043	1.36 ± .058	.98
	168-400	33	.128	1.06 ± .084	.91
Thio. sp. vs. bl. vol.	63-136	19	1.28	1.32 ± .171	.96
	115-400	42	4.53	.79 ± .051	.92
	63-400	52	2.67	.97 ± .038	.96
Pt. vol. vs. thio. sp.	63-400	52	.421	.82 ± .039	.95

'a' and 'b' are the constants in the equation $Y = aX^b$. S_b is the standard error of 'b'.
r = correlation coefficient.

The standard error of the estimated blood volume is rather large, about ± 9 per cent, although within a uniform group such as *group 6* it is considerably smaller as judged by inspection. The relatively large error, which may be due either to variation within animals or errors in the method, must be considered in any use to which blood volumes may be put.

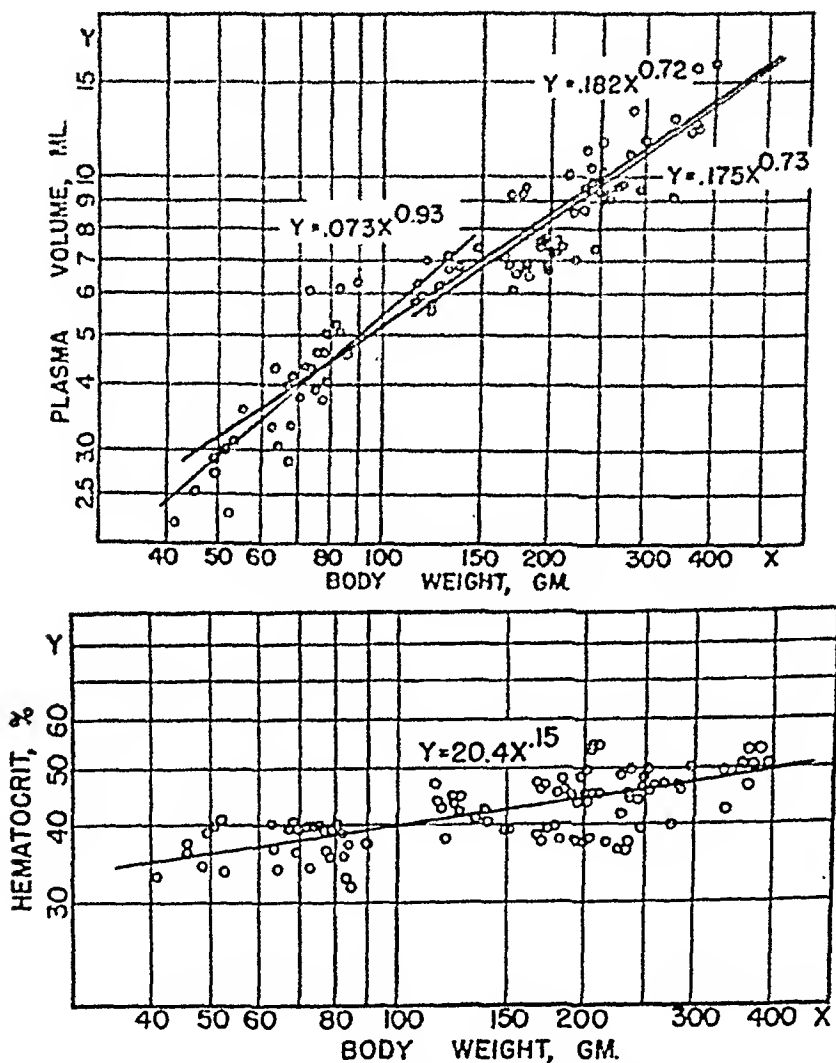


FIG. 2 (upper). RELATION of plasma volume to body weight in rats

FIG. 3 (lower). RELATION of the hematocrit to body weight in rats

A similar plot of the plasma volume data is shown in figure 2. In these data the inclusion of the group of animals weighing between 115 and 140 with the smaller animals improves the correlation somewhat and lowers the value of b from 1.17 to 0.93. Adding the data to that obtained from larger animals also improves the correlation and lowers the regression coefficient b from 0.80 to 0.72. It would thus appear that this weight range is where the break actually occurs, and the data may be included in either group. This was done in calculating the two regression lines shown in figure 2. It is of interest that the line obtained from the data from the adult animals is similar in slope to that obtained from the combined data of all the

animals. However, as pointed out with regard to Lippman's data, it is clear from inspection that this line does not fit at all well the plasma volumes of young rats. This line has a slope somewhat but not significantly less than that obtained by Lippman.

It is therefore concluded that the plasma volume of young rats may be best represented by the line $PV = .073 Wt^{.93}$, while in the larger animals, $PV = .175 Wt^{.725}$. As has been discussed with regard to blood volumes the weight range just above 100 gm. is in a transition zone, but it appears that either equation will give a fair estimate of plasma volume in this region while this was not true of blood volumes.

It should be noted that over either weight range the increase in blood volumes with body weight is more rapid than the increase in plasma volume. This difference, if it is real, must indicate an increase in the hematocrit with weight. As indi-

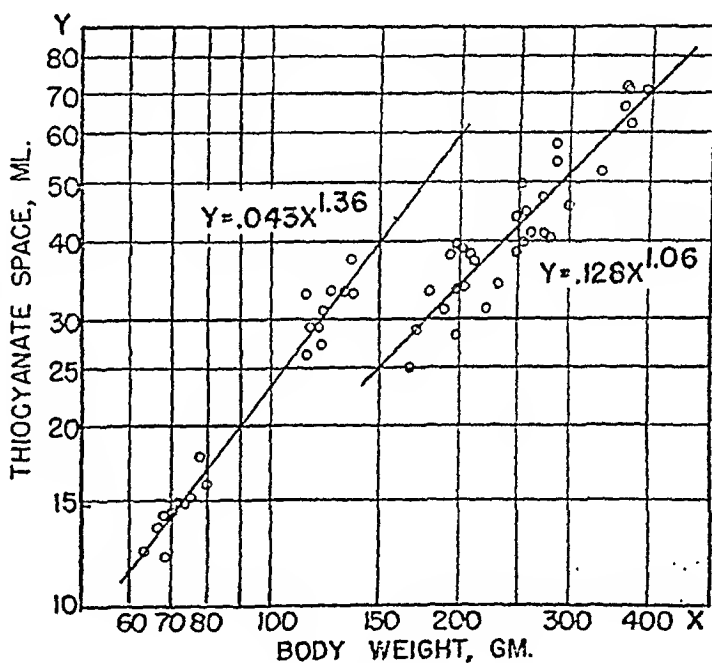


FIG. 4. RELATION of thiocyanate space to body weight in rats*

cated by the regression line in figure 3, there appears to be an overall increase of approximately 15 per cent in the hematocrit with a 100 per cent increase in body weight. It is impossible to tell whether there are breaks in this data comparable to those observed in blood and plasma volumes because of the relatively wide scatter within the animals, compared to the rather small increase with age. However, there is a suggestion of a break near 100-gm. body weight with a rather marked increase in hematocrit at about this age. Relatively little increase is apparent from 40 gm. up to 100 gm. This would account for the difference in the data observed in the blood volumes as compared to plasma volumes.

Since Metcalf and Favour did not determine thiocyanate space, the data available are considerably less numerous. However, as shown in figure 4 they are consistent with the previous findings in that thiocyanate space is apparently more accurately predictable with two regression lines than with one. In young animals the thiocyanate space apparently increases more rapidly than body weight, 136 per cent increase per 100 per cent increase in body weight. In adult animals the thio-

cyanate space increases nearly proportional to body weight. In these animals it is nearly constant at 17 per cent of the body weight.

In figures 5 and 6 the relation of thiocyanate space to blood and plasma volumes is shown. The overall change in blood volume is nearly proportional to thiocyanate space although there is a suggestion of a break in the data similar to that which has been shown previously. However, the plasma volume and thiocyanate space relation appears to be well represented by a single regression line for all of the animals. According to this line the plasma volume increases more slowly than the thiocyanate space. When the thiocyanate space increased 100 per cent the plasma volume increased 82 per cent. Thus the ratio $\frac{\text{thiocyanate space}}{\text{plasma volume}}$ is not constant but

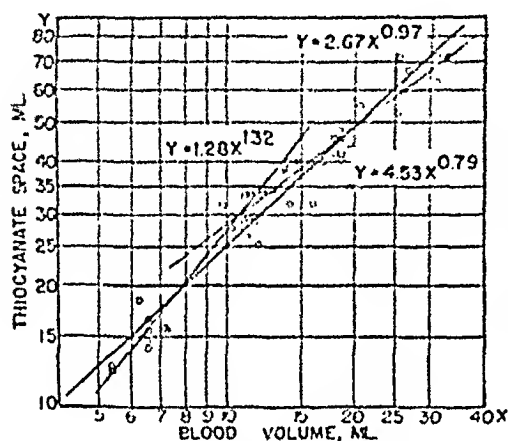


FIG. 5 (left). RELATION of thiocyanate space to blood volume in rats

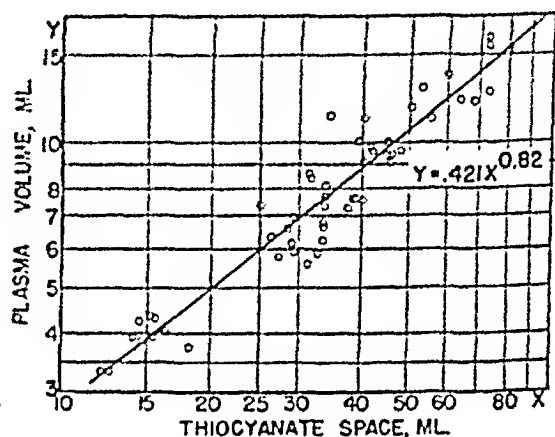


FIG. 6 (right). RELATION of plasma volume to thiocyanate space in rats

varies from approximately 3.9 for young rats weighing 60 gm. to approximately 4.8 for rats near 350 gm. in weight.

DISCUSSION

In the analysis of the data which have been obtained, we have attempted to determine the rate of change of the various body compartments with changes in body weight and with each other. This appears necessary rather than a simple statement of the values as percentage since there are apparent changes with age. Whereas blood volume can apparently be expressed as a percentage of body weight either in the young rats, 9 per cent of body weight, or in the adult animals, 7 per cent of body weight, this does not appear possible when thiocyanate space or plasma volume is being compared to body weight. In young rats the percentage of the body occupied by these fluids varies over a considerable range. In older animals thiocyanate space appears relatively constant when expressed as percentage of body weight.

While it is believed that the changes in the body fluid volumes in relation to body size as indicated in the figures and in table 2 are real, it would be unwise to attach too much significance to the absolute values obtained. As has been indicated,

even in the blood volume measurements which appear to be as reliable if not more so than most of the other determinations, the standard error of the estimate is of the order of 9 per cent. Thus two thirds of normal values should fall within ± 9 per cent of the predicted value and approximately 95 per cent within the region of ± 18 per cent. This obviously gives considerable spread to the data. How much of this spread is real in the sense that normal animals of the same strain and under similar dietary conditions vary in blood volume, and how much is due to errors in the method, are at present unknown. However the best that can be done with any method is to indicate a range within which normal animals fall.

In the experimental use of such data as has been obtained one wishes to be able to compare animals which have received various treatments, diets, etc. to some normal standard. Since the treatment may influence body weight some correction must obviously be made for this change. If adult animals are used the present data suggest that such adjustments could be made with a considerable degree of satisfaction. The situation would appear to be considerably more complicated when young animals are used, since marked changes in the various fluid compartments occur in our normal animals somewhere between 100 gm. and 140 gm. in weight when the animals are approximately 2 months of age. While it may be suspected that this change is related to sexual development, the weight or age at which it may occur may be expected to vary when the dietary or other experimental conditions cause changes in the rate of growth and development. If some of the animals reach mature weights while others in the same experiment with a different treatment grow poorly, it is doubtful if any valid comparison of their fluid volumes etc. can be made. Better experimental design would appear to be the use of adult animals, if possible, in which the relations of fluid volumes to body weight have become stabilized. It may be suggested also that the changes in metabolism, growth rate, food intake, organ size, etc. as related to body weight may have important implications which might well be considered in the planning of many experiments.

The actual data obtained agree reasonably well with those of Metcoff and Favour (1) and of Lippman (2). Our conclusions, however, differ. Whereas Lippman presented a single regression line as representative of animals of all weights, this does not appear valid for either blood volume or plasma volume. Two lines which have very similar slopes for blood volume give a more accurate picture of the relationship. Similarly two regression lines are required to show the relation of plasma volume to body weight, but these are apparently of different slope. Our data indicate a more important change in hematocrit, and thus less rapid increases in plasma volume than blood volume instead of essentially similar rates of increase with body weight as found by Lippman. With regard to the conclusions of Metcoff and Favour, we agree that the relation of plasma and blood volumes to body weight show a change at or shortly after puberty, but we find no valid reason for calculating either blood or plasma volumes in terms of surface area. Since surface area is calculated as a power of body weight, it is more logical to use the actual power of body weight found experimentally rather than an arbitrary one such as $Wt^{.6}$. Kleiber (9) has fully discussed the errors and inconsistencies involved in the use of surface area as a unit in metabolic studies. The same reasoning is applicable here.

SUMMARY

Normal values of blood and plasma volume and of thiocyanate space were determined in rats ranging in weight from 40 to 400 grams. The relation of these fluid volumes to body weight appears to change markedly at or shortly after puberty. Prior to this time the blood volume appears to be proportional to the body weight (varies as $Wt^{1.0}$), the plasma volume increases at a slightly lower rate with increases in body weight (varies as $Wt^{.92}$) and the thiocyanate space increases more rapidly than body weight (varies as $Wt^{1.3}$). In adult animals the blood volume is lower but again increases in proportion to the body weight, the plasma volume increases with body weight at a considerably slower rate (varies as $Wt^{.72}$) and thiocyanate space varies approximately in proportion to body weight (Wt^1).

The changes in hematocrit with weight, increase in proportion to $Wt^{.15}$, apparently account for the different relations of blood and plasma volumes to body weight.

Thiocyanate space and plasma volume relation is apparently reasonably constant over the entire weight range studied. The plasma volume increases in proportion to thiocyanate space^{.62}. Thus the ratio of thiocyanate space to plasma volume falls with increasing size of the animals.

We are indebted to Merck & Company, Inc., Rahway, N. J., Corn Industries Research Foundation, New York City, Gaines Division of General Foods Corporation, Hoboken, N. J., Sheffield Farms Company, Inc., New York City, Eli Lilly & Company, Indianapolis, Ind., and William R. Warner Company, New York City, for generous supplies of materials used in these studies.

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DETERMINATION OF BLOOD AND PLASMA VOLUMES, THIOCYANATE SPACE, AND BROMSULFALEIN CLEARANCE IN RATS¹

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ALTHOUGH the rat is a convenient animal and has been used extensively in nutritional studies with proteins, there are relatively few reports of the changes in the various fluid compartments of the body (blood volume, plasma volume, and thiocyanate space) during protein depletion in this species. In general, the methods which have been used for the measure of the space occupied by these fluid volumes require too much blood to allow repeated estimates in animals as small as the rat (1-7). Protein deficiency is also known to produce marked histological changes in the liver (8, 9) and the measurement of changes in liver function might also be of use in evaluating the effects of protein depletion. Again, most of the methods available require considerable blood and have not been adapted for use with small animals.

A series of studies has been completed in which changes in blood and plasma volume, thiocyanate space, bromsulfalein retention, and the chemical and histological changes in the liver have been measured at intervals during protein depletion in rats. The results obtained will be reported in subsequent papers. This paper describes the methods used for the determination of blood and plasma volume, thiocyanate space and bromsulfalein clearance in rats which were done with one injection of a mixed solution of Evan's Blue Dye (T-1824), thiocyanate and bromsulfalein. Two small blood samples, 0.5 ml. each, were drawn at 5- and 10-minute intervals after the injection. These suffice for the measure of the hematocrit and the content in the blood of the three materials injected.

METHODS

Animals ranging from 60 to 400 gm. in weight were used. The methods were uniform except that in order to achieve blood concentrations of a convenient range, the amount of the dye T-1824 and the amount of thiocyanate injected were decreased to half the amount stated below when animals below 100 gm. in weight were used.

Injection and Blood Sampling. With the animal under light ether anesthesia, the front of the neck was washed with soap and water, shaved, and washed with alcohol. An incision about 2 cm. long was made from the sternal end of the clavicle

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to the angle of the lower jaw, and the fascia separated by blunt dissection to expose the jugular vein. The foreleg on the same side as the incision was fixed in a suitable position with a push pin to expose the vein prominently. At zero time, 0.4 ml. of a solution containing 5 mg. bromsulfalein, 10 mg. sodium thiocyanate, and 0.9 mg. Evan's Blue was injected with a calibrated tuberculin syringe. The needle was left *in situ* for about 10 seconds until all of the dye had left the vein. At exactly 5 and 10 minutes after injection, 0.5 ml. of blood was taken by heart puncture with a sterile syringe previously moistened with heparin solution (Liquacmin). With practice, the samples could be obtained almost exactly at the correct time and the needle was inserted slightly in advance to prevent delay if the heart was not punctured immediately. However, the time did not need to be exact except for the liver function test, and even there the results suggested that adequate corrections could be made for variations in time.

Four-tenths ml. of blood was diluted 10 times by adding it to a small tube containing 3.6 ml. of normal saline, mixed gently, and centrifuged for 15 minutes. The supernatant was used for the various determinations. A sample of blood obtained from a control, non-injected animal was similarly treated for use as a blank in the various measurements. The remaining blood which had not been diluted was used for measure of the hematocrit. We used Van Allen tubes spun for one hour at 3000 rpm in this determination.

Blood and Plasma Volumes. Two ml. of the supernatant was placed in small standardized colorimeter tubes and read in the colorimeter equipped with the 575 millimicron filter and set to allow 100 per cent transmission with the control sample. The concentration of Evan's Blue was determined from a previously constructed standard curve, and the amount of dye in the 2-ml. sample calculated. Since this amount of dye was obtained from 0.2 ml. of blood, the blood volume was readily obtained as $\frac{\text{mg. injected}}{5 \times \text{mg. in sample}}$. Plasma volume was calculated as the blood volume times 1-hematocrit.

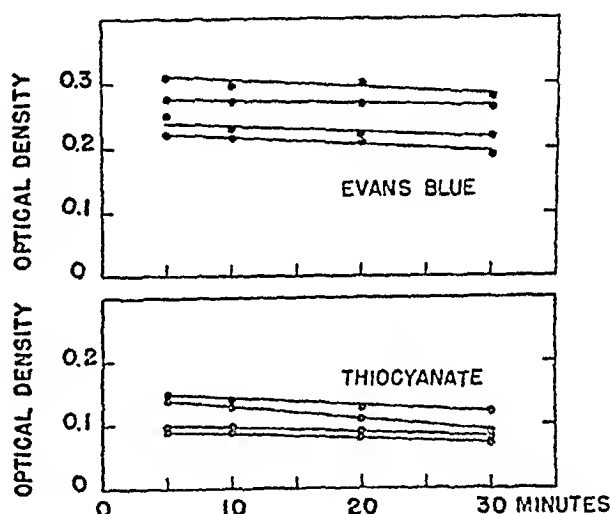
Bromsulfalein Determination. The color of the bromsulfalein was developed by the addition of 0.01 ml. of 10 per cent sodium hydroxide to the tube previously read in the colorimeter and immediately read again using the same filter. The value obtained was due to the sum of the color of the Evan's Blue and the bromsulfalein, and bromsulfalein alone was obtained by difference since the Evan's Blue reading was not changed by the alkali.² In order to make this subtraction, the readings were converted into optical densities (2-log. reading). The optical density thus obtained for the bromsulfalein alone was then compared to a standard curve to obtain the concentration in the sample and the concentration in the original blood calculated. The

² The absorption spectrum of the Evan's Blue-protein complex is changed and shifted to the right by the addition of alkali, but the transmission of the 575 m μ filter in the Lumetron colorimeter is such that the reading is essentially unchanged by this shift. In adapting the method to the Beckman spectrophotometer it was found that equal transmission before and after alkali was obtained at 581 m μ and standard curves were therefore made using this wave length. One may, of course, read the Evan's Blue at another appropriate wave length and make a calculated correction of the reading for bromsulfalein. This will be necessary if a selected filter does not give the same reading before and after the addition of alkali to the Evan's Blue plasma solution.

amount cleared from the blood was then calculated as the difference between the dose injected and the total amount in the blood as determined from the concentration and the blood volume.

Thiocyanate Space. The thiocyanate content of the supernatant was determined with another sample from the 10-minute blood sample. One ml. of the supernatant and an equal volume of 20 per cent trichloroacetic acid were mixed and allowed to stand 10 minutes. After centrifugation, 1 ml. was placed in the photometer tube, followed by 1 ml. of 5 per cent ferric nitrate solution. The tube was immediately read using the 420 millimicron filter with the instrument set to give 100 per cent transmittance with the control tube similarly treated, and the thiocyanate content determined from a standard curve. The sample read in the colorimeter represented 0.05 ml. of blood. The thiocyanate space was obtained by dividing the dose injected by the thiocyanate content per ml. of blood.

FIG. 1. EVAN'S BLUE DYE and thiocyanate concentration in the blood at intervals after the time of injection.



According to Lavietes *et al.* (7) thiocyanate distributes itself in the plasma and red cells in approximately the same concentration. This was studied by comparing the thiocyanate content of whole blood and plasma obtained from the same samples of blood. In 16 different bloods which contained approximately 180 μg . of thiocyanate per ml., the mean difference in concentration of the plasma and blood was only 1.5 $\mu\text{g}/\text{ml}$. This was well below the accuracy of the methods involved. Thus in our experience, the distribution in plasma and cells appeared to be practically the same.

Mixing Time of Evan's Blue and Thiocyanate. The results obtained by sampling at intervals after injection on the Evan's Blue and thiocyanate concentration of the blood are shown in figure 1. In both instances, a relatively constant concentration appeared to have been obtained after 5 minutes. However, we used the 5-minute sample for the calculation of blood volume and the 10-minute sample for thiocyanate space. No attempt was made to correct the values obtained to zero time.

Bromsulfalein Liver Function Test. The rate of disappearance of bromsulfalein from the blood in relation to time was investigated in a group of adult animals all of approximately the same weight. As shown in figure 2 the dye disappeared from the

blood at a rapid but decreasing rate, and a straight line is obtained if $\frac{1}{\text{blood conc.}}$ is plotted against time. This relation may be expressed also as concentration \times time = a constant, and correction may be made to obtain the standard 5-minute concentration by substitution of the concentration and time, calculation of the constant, and then solving for the concentration when time = five minutes.

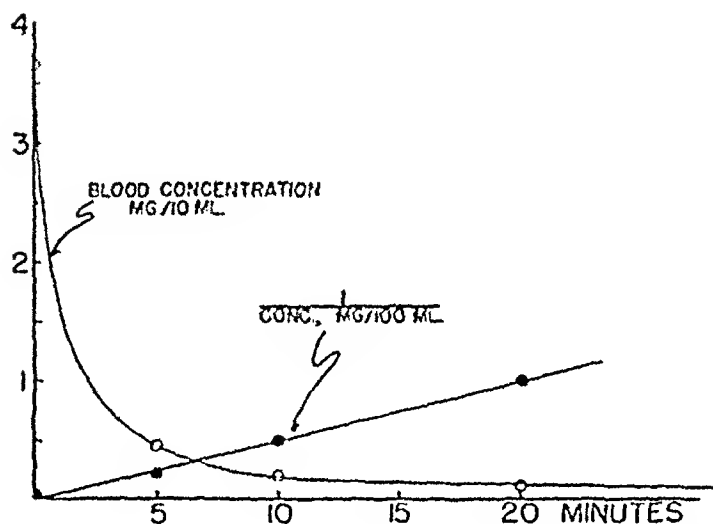
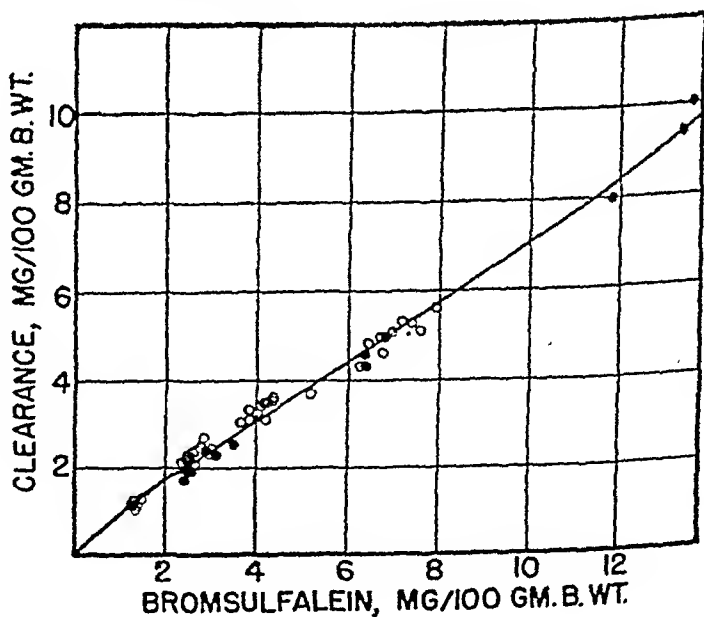


FIG. 2. GRAPH SHOWING THE BLOOD CONCENTRATION of bromsulfalein at various times after injection, and the straight line relation obtained by plotting $\frac{1}{\text{concentration}}$ against time. The original concentration was calculated from the blood volume and the dose injected. The concentration was expressed in different units to allow plotting upon the same scale.

FIG. 3. TOTAL CLEARANCE OF BROMSULFALEIN after 5 minutes compared to dose of bromsulfalein. Open circles indicate animals which received the same dose, 5 mg. Solid circles indicate adult animals which received 5, 10, or 20 mg.



In order to account for differences in body size, the clearance in mg/100 gm. of body weight was calculated using the blood volumes and the blood concentration. The clearance, as shown in figure 3, is directly related to the dose of bromsulfalein also expressed as mg/100 gm. of body weight. Most of the points on the curve were obtained by injecting the same dose of 5 mg. of bromsulfalein in rats of different sizes, but the few points obtained by injecting different size doses in animals weighing about 200 gm. fit the same curve even at extremely high levels.

DISCUSSION

The methods described have been used extensively with excellent results in rats as will be shown in subsequent papers. The calculation of the bromsulfalein test in terms of clearance based upon the actual blood volume would appear to have both practical and theoretical advantages over estimated values. Since the plasma volume, thiocyanate space and liver function may be determined following the injection of one solution with practically no additional work, other than a few manipulations and colorimeter readings, over that ordinarily required for the measurement of any one of these tests alone, the extension of these methods to clinical practice would bear investigation. Undoubtedly the combined data would be more useful clinically than any of the single determinations. While the use of small blood volumes was of importance in these studies with small animals, the volumes may, of course, be varied to any convenient range.

The application of the ordinary clinical liver function tests for use in small animals should allow studies not possible with clinical material. However, it may be noted that the ability of the rat to remove bromsulfalein from the blood far exceeds that of the normal human being per unit body weight. The dose of bromsulfalein which we have used routinely in rats is at least 10 times per kilo of body weight that used in clinical practice. Nevertheless, it is apparent from the fact that the clearance is a constant function of dosage even at much higher levels, that the dose is far below the maximum ability of the animal to remove the dye, and presumably little stress is actually placed upon the liver by such dosages. The same appears to be true in the usual clinical test since normal rates of removal are obtained when a considerable part of the liver is apparently non-functional. The shape of the disappearance curve indicates that the method loses accuracy when the time interval is unduly prolonged since the rate of change becomes extremely small as the blood concentration reaches low levels.

SUMMARY

Methods have been described for the simultaneous determination of blood and plasma volume, thiocyanate space and bromsulfalein clearance which are suitable for use with rats. In agreement with previous investigators, Evan's Blue dye utilized for the determination of blood and plasma volumes was uniformly distributed in the blood before the lapse of 5 minutes after injection. Thiocyanate also appears to have been uniformly distributed after 5 minutes although we allowed 10 minutes as a matter of precaution.

Rats have a great ability to remove bromsulfalein. In rats of the same size (equal blood volume), the rate of removal from the blood during the first 20 minutes after injection is such that $\text{concentration} \times \text{time} = \text{a constant}$; correction may thus be made for variations in the time when blood samples are taken. With rats ranging from 60 to 400 gm. in weight, the clearance from the blood per 100 gm. of body weight was found to be directly related to the dose per 100 gm. of body weight.

We are indebted to Merck & Co., Inc., Rahway, N. J., Corn Industries Research Foundation, New York City, Gaines Division of General Foods Corporation, Hoboken, N. J., Sheffield Farms Company, Inc., New York City, Eli Lilly & Company, Indianapolis, Ind., and William R. Warner Company, New York City, for generous supplies of materials used in these studies.

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SELF-SELECTION OF SALT SOLUTIONS AND WATER BY NORMAL AND HYPERTENSIVE RATS

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THE tendency of animals to regulate their intake of food and water according to their metabolic requirements has been observed in pigs (1), domestic fowl (2) and normal rats (3). More recently Richter (4) has reviewed many examples of the striking specificity of such 'self-selection' on the part of rats made abnormal through adrenalectomy, hypophysectomy, parathyroidectomy or pancreatectomy. When offered those salts or foods which might counterbalance an induced abnormality, such animals generally chose very accurately those materials which tended to compensate for specific malfunction and thus prolonged life.

For experimentally hypertensive rats little is known concerning such self-selection except for their tendency to take more water than control animals (5). It seems possible that, if hypertensive rats were given systematically a choice of a wide variety of salts and foods, changes in their blood pressure together with their voluntarily reduced or increased intake of one or more specific substances might indicate metabolic abnormalities associated with the hypertensive state.

This paper describes studies on normal and hypertensive rats indicating *a*) the range of variation in the self-selective intakes of certain isosmotic salt solutions, *b*) the lowered intake of NaCl and NaHCO₃ by hypertensive rats and *c*) the unchanged intake of certain salts which sometimes alleviate hypertension of man or animals.

METHODS

White male rats of the Rockland or Wistar strains, weighing between 150 and 185 gm., were first observed for 1½ to 3 weeks, their blood pressures being measured three or more times in this period by means of the heated plethysmograph described by Sobin (6). Those with systolic blood pressures read once above 140 mm. Hg, or with average values above 130 mm. Hg, were discarded to exclude any rats with a possible tendency toward spontaneous hypertension. Of the remainder, from one half to one third were kept as controls, while the others were subjected to bilateral renal encapsulation, using the latex rubber molded sheaths described by Abrams and Sobin (7). The rats which developed hypertension (usually 70-90% of those operated and surviving) were divided into two groups, viz. 'moderately hypertensive' with systolic blood pressures between 160 and 185 mm. Hg and 'severely hypertensive' with systolic blood pressures between 185 and 240 mm. Hg.

Groups of 3 rats, each in a 20" x 12" x 10" cage, were offered distilled water and five salt solutions by means of six drinking bottles, three on each side of the cage, with tips shaped as small bowls to prevent spillage and to reduce evaporation to negligible amounts (less than 0.1 cc/day). Bottles and tips were cleaned carefully and refilled daily to assure an unfailing supply of solution. To avoid habit formation or conditioning, the positions of all six bottles were changed daily. The bottoms of the cages consisted of half-inch mesh, permitting feces and urine to fall to a pan beneath, thereby

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avoiding errors from coprophagy and drinking of urine. Salts were offered in 0.17 molar solutions in distilled water except for special observations designated below. The amount of each solution taken by 3 rats was determined by daily weighing of the bottles. To prevent confusion with grams of solids taken, the intakes of fluids are given in cc., assuming their specific gravity equals unity. The error in this assumption is less than 1.0 per cent and, being consistent, does not affect the validity of the comparisons made.

In most experiments, 18 rats were followed concurrently, viz. two cages each containing 3 normal rats, two cages each containing 3 moderately hypertensive rats and two cages each containing 3 severely hypertensive rats. Whenever possible, additional or 'spare' control and hypertensive animals were placed on each experimental regimen so that if illness or death reduced the number in a given experimental cage, another rat in the same category might be shifted at once to that cage to prevent interference with the standard observation.

As each new group of six solutions was presented, the rats tended for the first few days to take widely varying amounts of some solutions but, after 2 to 10 days, daily intakes became fairly constant and choices or aversions became clearer. Nevertheless, because of day-to-day variation, even after the preliminary stabilization period of 2 to 10 days, all conclusions have been based, with rare exceptions, upon a minimum period of 30 rat days, i.e. mean intake of 3 rats for 10 days, in terms of the volume of solution taken per day (cc/100 gm. of rat/24 hr.). In comparing intakes, the standard errors of the means and the 't' values were calculated to determine the significance of any differences observed during the last 10 days of each total 12-to-20-day period of test.

Body weights and systolic blood pressures were recorded twice weekly. Changes in the general appearance, activity and food intake were noted daily. Each of the cages contained four plastic, angled, tubular food cups mounted in the back wall so that daily food intake could be measured accurately without loss. Diet during the experimental periods consisted in most instances of powdered Purina Laboratory Chow, containing, according to the manufacturer, 0.4 per cent Na as NaCl. For comparison in the initial series of salts, a salt-poor synthetic diet was used in one series as follows:

Sucrose.....	73 gm.	Corn oil.....	5 gm.
Purified casein.....	18 gm.	Salt mixture.....	4 gm.

The specific salts being tested in each experiment were omitted from this mixture which otherwise was as given by Hegsted *et al.* (8).

Thiamine chloride.....	0.3 mg/100 gm. ration
Riboflavin.....	0.4 mg/100 gm. ration
Pyridoxine hydrochloride.....	0.3 mg/100 gm. ration
Calcium pantothenate.....	1.0 mg/100 gm. ration
Choline chloride.....	100.0 mg/100 gm. ration
Vitamins A and D, each.....	25.0 U./100 gm. ration

OBSERVATIONS

Selection of Common Cations, Series A. The results observed for 3 normal, 3 moderately hypertensive and 3 severely hypertensive rats in a typical 10-day period offering 0.17 M. solutions of NaCl, KCl, CaCl₂, MgCl₂, NH₄Cl and water are shown in figure 1. Despite considerable daily variation it appears that the hypertensive rats took *a*) less NaCl solution and *b*) more distilled water than the normal rats did, but drank smaller and equal amounts of the KCl, CaCl₂, MgCl₂ and NH₄Cl solutions. Even though each solid bar represents the average intake by 3 rats, daily variations were great enough to require statistical analysis to determine the significance of differences between means for each 10-day period.

Figure 2 illustrates the collective results of four experiments (120 rat days) similar to that in figure 1. In this and most subsequent figures the heavy bars (to

² A more recent manufacturer's analysis reports 0.97 per cent Na.

SOLUTIONS A — UNIT OBSERVATION — 3 RATS, 10 DAYS

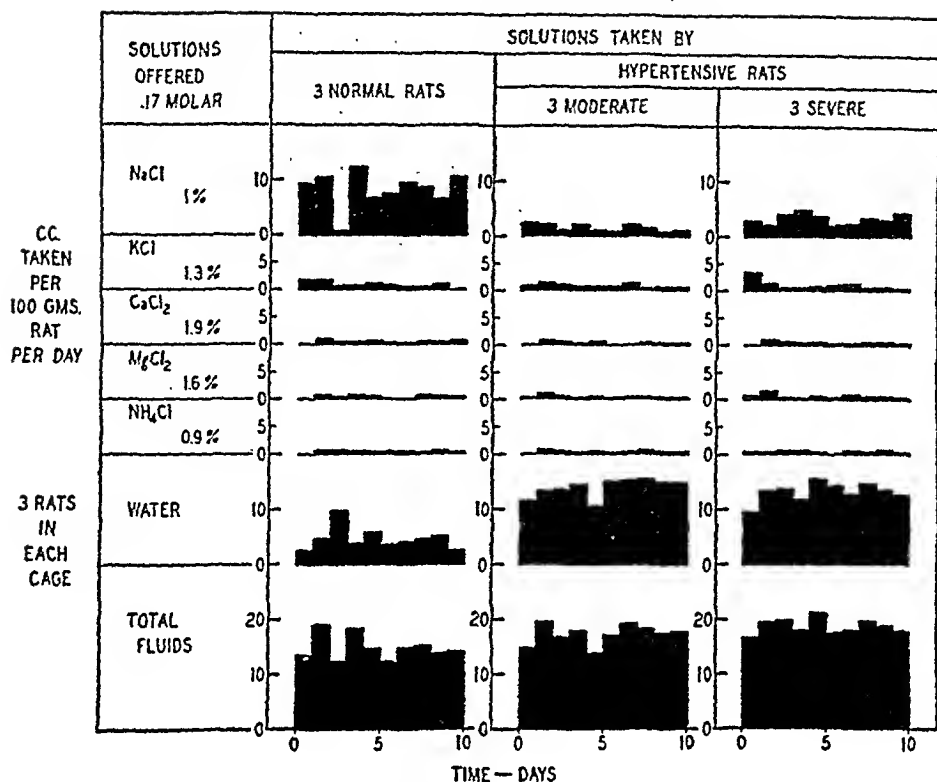


Fig. 1. CHART SHOWING DAILY INTAKES of water and of salt solutions by normal and hypertensive rats for 10 days, in groups of 3 rats each.

SOLUTIONS A







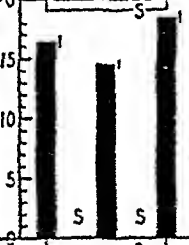
SALT SOLUTIONS CC. OF .17 MOLAR	INTAKE - CC. PER 100 GMS. BODY WEIGHT PER 24 HOURS MEAN AND STANDARD ERROR OF MEAN						BASED ON RAT-DAYS NUMBERING
	NOR- MAL	HYPERTENSIVE		NORMAL	HYPERTENSIVE		
		MOD.	SEV.		MODERATE	SEVERE	
NaCl		6.27 ±.54	2.39 ±.31	4.48 ±.36	120		
KCl		.79 ±.11	.66 ±.10	.66 ±.11	120		
CaCl ₂		.21 ±.04	.24 ±.04	.28 ±.04	120		
MgCl ₂		.24 ±.04	.40 ±.09	.30 ±.05	120		
NH ₄ Cl		.28 ±.04	.32 ±.05	.35 ±.06	120		
WATER		8.61 ±.16	10.52 ±.43	12.28 ±.35	120		
TOTAL FLUIDS		16.40 ±.45	14.53 ±.37	18.35 ±.48	120		

Fig. 2. SUMMARY OF INTAKES, salt solutions of Series A, each bar representing av. for 120 rat days. For description, see text.

the left) indicate mean daily intake of each salt solution in cc/100 gm. body weight; the thin lines to the right of the tops of the bars represent the standard error of the corresponding means. A capital 'S' between bars indicates that the difference between the two corresponding means is highly significant whether calculated from the standard errors of the means ($M_1 - M_2 > 2 \sqrt{S.E._{M_1}^2 + S.E._{M_2}^2}$) or by Fisher's

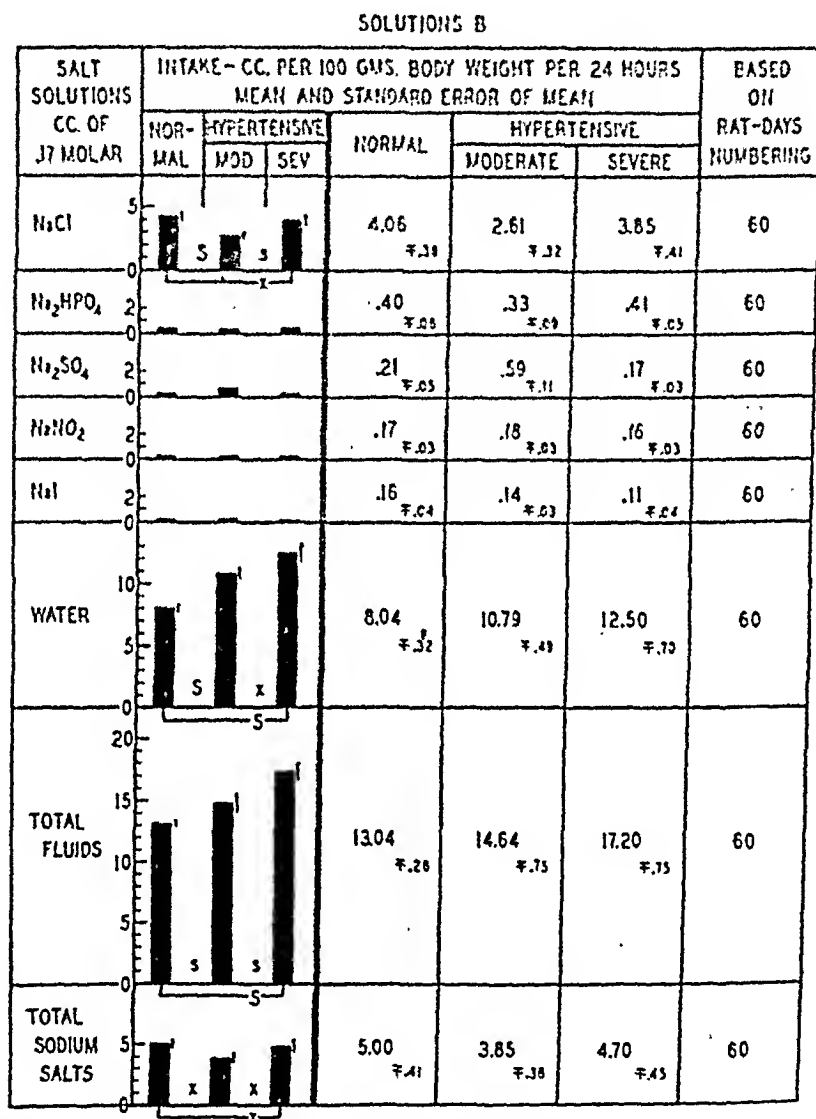


Fig. 3. SUMMARY OF INTAKES, salt solutions of *Series B*, each bar representing av. for 60 rat days. For description, see text.

't' test (P is .01 or less). A small 's' between bars indicates that ' P ' lay between .05 and .01 and that the difference is questionably significant. Absence of the letter 'S' or presence of an 'X' indicates that the means were not significantly different. For more detailed comparisons the table in the right portion of figure 2 gives the numerical means on which the bars are based, together with the standard error of each mean.

From figure 2 it seems clear that moderately hypertensive rats elected to take significantly less one per cent NaCl solution than did normal rats and that their

intake of water was significantly greater. The severely hypertensive rats while taking most water, took more NaCl than the moderately hypertensive rats, though still less than the normal rats. This change of NaCl intake in moderate and severe hypertension was found consistently as shown also in figures 3 and 4. In agreement with previous reports (5), the severely hypertensive rats took most water.

Of the other salts, KCl, CaCl_2 , MgCl_2 and NH_4Cl , much smaller and equal amounts were taken and without any significant differences between the intakes of normal and hypertensive animals.

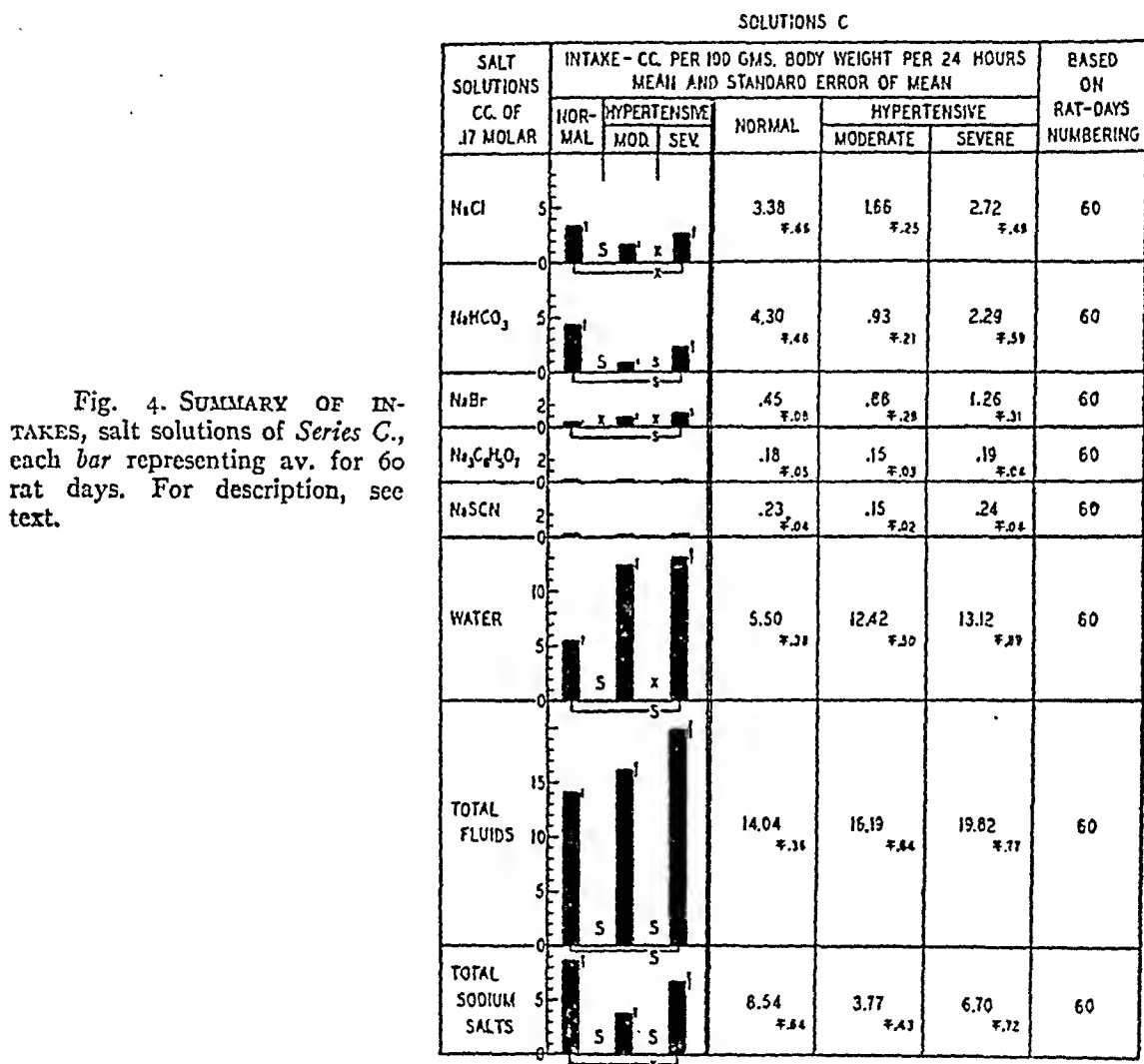


Fig. 4. SUMMARY OF INTAKES, salt solutions of Series C., each bar representing av. for 60 rat days. For description, see text.

Selection of Certain Anions Combined with Sodium, Series B and C. In addition to the common anions, e.g., HPO_4 , SO_4 , HCO_3 and citrate, these groups contained salts that have been used in the symptomatic treatment of hypertension or arteriosclerosis, e.g., NaNO_2 , NaBr , NaSCN and NaI (figs. 3 and 4). These were offered to determine *a*) whether or not any of them would be taken selectively by hypertensive rats and *b*) whether or not they would affect either the hypertension or the voluntary restriction of NaCl.

As in the previous series, the moderately hypertensive animals took least NaCl

solution (figs. 2 and 3) and more water. The severely hypertensive animals took only slightly less NaCl solution than the controls though their water and total fluid intake were greater. Differences were less striking in *Series B* (fig. 3) when choice included five sodium salts, viz., NaCl, Na_2HPO_4 , Na_2SO_4 , NaNO_2 and NaI, than in *Series A* (fig. 2) in which only one sodium salt was offered. This difference may have been due to a 'tasting error' which will be described in greater detail below. It may be presumed that when several equally concentrated solutions of sodium salts were offered, mere sampling tended to produce a smaller, and possibly less consistent, intake of NaCl, because all the offered solutions contained Na ions. This explanation is supported *a*) by the still less clear difference between normal and hypertensive rats when total intake of all sodium salts was computed by addition of the means (bottom fig. 3) and *b*) by the observation that small amounts of salts other than NaCl were taken only sporadically, whereas on many days intake was zero or so near zero as not to be detectable in weighing the bottles to the nearest gram. NaCl, on the contrary, was taken every day in considerable and more uniform volumes.

When offered a choice between NaCl and NaHCO_3 (*Series C*, fig. 4), specificity of aversion and choice was again surprisingly clear. Sodium intake was partitioned chiefly between these two solutions, while the others were neglected almost completely. In this series, intake of NaCl, NaHCO_3 and of total Na salts was least in the presence of moderate hypertension, with the same tendency on the part of severely hypertensive animals to increase their intake slightly.

For the other ions, HPO_4 , SO_4 , NO_2 , I, SCN and citrate, intakes (figs. 3 and 4) were minimal and equal in all groups, with the single exception of NaBr for which a doubtfully significant increase of intake appeared in the severely hypertensive group. More amusing than important was the observation that when hypertensive animals had access to NaBr for a long time, e.g., 38 days, some, but not all, took enough accumulated bromide to become somnolent or even semi-stuporous. Their food intake fell off, they lost weight and their blood pressures in some instances fell toward normal. Removal of NaBr led almost at once to restoration of activity, food intake, body weight and hypertension. In agreement with the doubtful significance of the differences between the mean intakes of NaBr in figure 4 this sequence was observed in only some hypertensive animals and even in them not consistently.

Comparison of Sodium Intake with Purina Chow and with a Purified Synthetic Diet. To exclude the possibility that these findings were due to the composition of the accompanying diet of Purina Chow, the observations were all repeated in alternate periods using (fig. 5) the purified diet described under METHODS. As each series of salts was given, the salts offered in solutions were omitted from the salt mixture which formed part of the diet corresponding to each period. All rats, normal and hypertensive, took less of the synthetic diet than of Purina Chow and their body weights did not increase as rapidly as they did on the latter. The purified diet contained more water so that average total fluid intake was less despite the development of mild diarrhea by some animals. Nevertheless, on this diet also, as shown in table 1, the moderately hypertensive rats took least NaCl, NaHCO_3 and total sodium salts. While this relative difference persisted, it is interesting that the *absolute* volumes of NaCl, NaHCO_3 and total Na solutions taken were generally

greater (one minor exception) on the synthetic diet than on Purina, as might be expected because the former diet contained much less sodium than did the latter.

The remaining salts were taken in similar and minute amounts by all 18 rats, normal and hypertensive, with the single exception of NaBr, of which the severely hypertensive rats again took almost three times as much as the controls. Water intake was generally less but the relative polydipsia of the severely hypertensive rats was also evident on the purified diet though at a somewhat lower absolute level. It was concluded that the observed differences in the intakes of sodium chloride and bicarbonate were not due to the diet given concurrently.

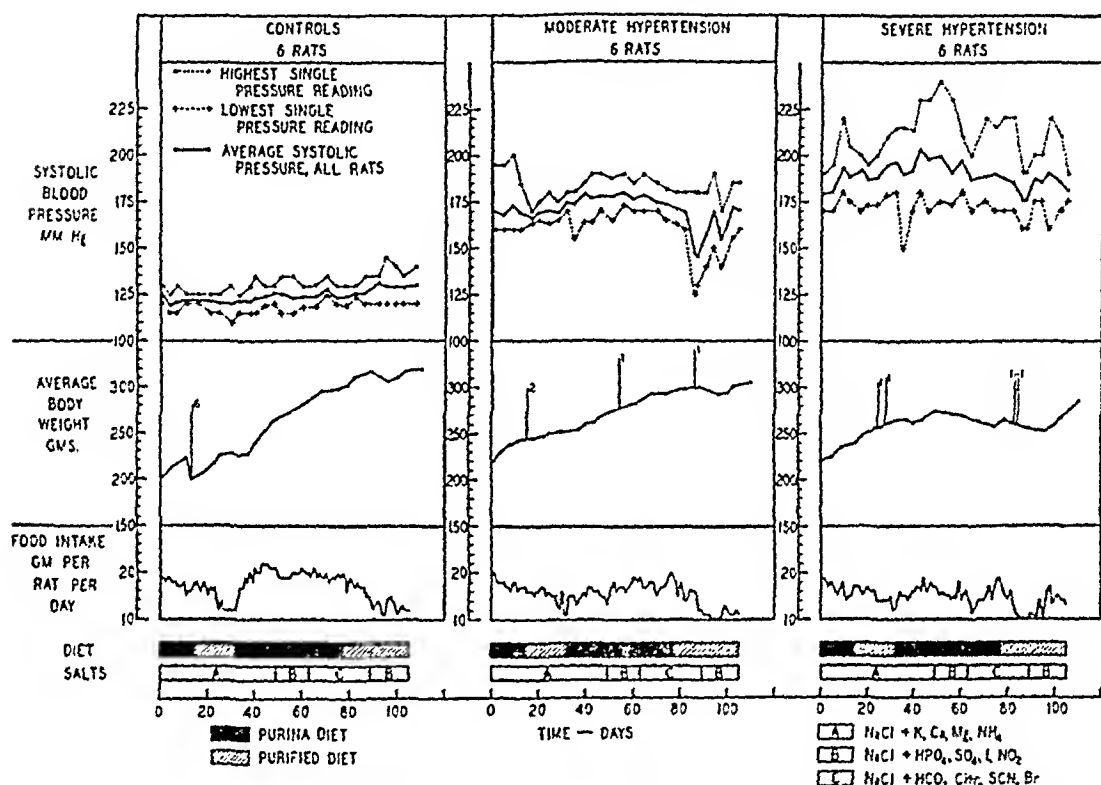


Fig. 5. CHART SHOWING systolic blood pressures, body wt. and food intakes while offered 3 series of salt solutions and 2 diets.

Food Intake, Body Weight and Blood Pressure. These are summarized in figure 5 in which the shaded blocks indicate the diet offered and the lettered blocks the salts offered. The salts corresponding to the letters are shown in the lower right hand corner of the figure. The vertical lines on the curve of body weight (fig. 5) indicate substitutions of animals from the spare stock mentioned under METHODS. The whole control group had to be changed early in the experiment because of diarrhea. Single hypertensive animals had to be substituted occasionally as shown because of cardiac failure or cerebral hemorrhage.

Food intake was greatest and most constant when Purina Chow was offered, least and most variable when the synthetic diet was used. Body weight also increased most rapidly when Purina Chow was given, but the over-all gain in weight was generally least in the severely hypertensive group.

Systolic blood pressure remained fairly constant throughout except *a*) for the normal slight rise in the control group as they became older and *b*) a transient fall in the moderately hypertensive group when the intake of NaBr led to stupor of some animals and reduction of food intake almost to zero. It is noteworthy that though the severely hypertensive animals took more NaBr and were equally stuporous with almost zero food intake, their blood pressures remained high. There was no evidence at any time that the reduced intake of NaCl reduced blood pressure presumably

TABLE 1. CHOICES OF SALT SOLUTIONS AND WATER ON SYNTHETIC DIET (ALL FOR 60 RAT DAYS).
STANDARD ERRORS OF MEANS COMPUTED ONLY FOR NaCl AND NaHCO₃

SOLUTIONS (SALTS 0.17 M.)	INTAKE, CC/100-GM. BODY WT/24 HR.		
	Normal	Hypertensive	
		Moderate	Severe
NaCl	8.27 \pm 0.66	5.11 \pm 0.56	5.88 \pm 0.46
KCl	.72	.87	1.15
CaCl ₂	.22	.22	.31
MgCl ₂	.28	.31	.29
NH ₄ Cl	.41	.26	.38
Water	2.73	4.15	6.25
NaCl	5.87 \pm 0.19	4.43 \pm 0.45	8.31 \pm 0.51
Na ₂ HPO ₄	.22	.24	.30
Na ₂ SO ₄	.31	.49	.24
NaNO ₂	.27	.27	.28
NaI	.26	.27	.29
Total Na	6.93	5.70	9.42
Water	2.46	5.94	7.16
NaCl	2.32 \pm 0.22	3.06 \pm 0.29	4.07 \pm 0.59
NaHCO ₃	5.94 \pm 0.45	1.40 \pm 0.18	2.22 \pm 0.35
NaBr	.40	.52	1.10
Na citrate	.22	.24	.24
NaSCN	.24	.24	.20
Total Na	9.12	5.46	7.83
Water	1.23	5.77	6.78

because voluntary restriction was not sufficiently complete to be effective to the degree that a rigidly salt-poor diet is (Grollman and Harrison, 9).

Consistency of Sodium Intakes by Different Strains of Rats. The comparisons so far described were carried out on rats of a single strain (Sherman) and source (Rockland Farm). Table 2 summarizes data on three separate lots of the Sherman strain and four lots of the Wistar strain, the latter coming from two sources, Wistar Institute and the Charles River Animal Farm. Whether the results are considered as individual groups or in terms of grand means, it seems clear that the hypertensive animals of all lots and both strains consumed less Na, in the form of NaCl and NaHCO₃, than did normal animals. The differences appear to be greater in the Wistar strain, but this may have been due to the smaller number of Wistar animals studied.

Comparison of Intakes by Single Rats and by Groups of Three. To provide larger statistical samples, the observations described above were based on minimum unit periods of 30 rat days, i.e., 3 rats together in one cage observed for 10 days after stabilization on each group of choices. It was expected that in exploratory studies such pooled figures would reveal trends more uniformly and rapidly than if single rats were used. That this was the case is shown by figure 6.

In this comparison 9 rats, viz., 3 controls and 6 moderate hypertensives, were placed in single cages with choice of water and 0.17 M. solutions of NaCl and

TABLE 2. COMPARISON OF TOTAL SODIUM INTAKES BY DIFFERENT STRAINS AND GROUPS OF RATS. STANDARD ERRORS OF MEANS INCLUDED WHERE THEY WERE COMPUTED IN THE COURSE OF OTHER ANALYSES

STRAIN AND GROUP NO.	INTAKE, TOTAL Na SOLUTIONS CC/100 GR. BODY WT/24 HRS.			TOTAL RAT DAYS			SALT SOLUTIONS OFFERED 0.17 M.
	Normal	Hypertensive		Nor.	Mod.	Ser.	
		Moderate	Severe				
<i>Sherman</i>							
1	6.27 ± .54	2.39 ± .31	4.48 ± .38	120	120	120	Series A, fig. 2
	5.00 ± .41	3.85 ± .38	4.70 ± .45	60	60	60	Series B, fig. 3
	8.54 ± .64	3.77 ± .43	6.70 ± .72	60	60	60	Series C, fig. 4
	8.27 ± .66	5.11 ± .56	5.88 ± .46	60	60	60	Series A, table 1
	6.93	5.70	9.42	60	60	60	Series B, table 1
	9.12	5.46	7.83	60	60	60	Series C, table 1
2	5.67 ± .51	2.12 ± .34	2.47 ± .57	60	30	30	NaCl, NaHCO ₃ , H ₂ O
3			4.79 ± .83			60	NaCl, NaHCO ₃ , H ₂ O KCl, NaBr, MgCl ₂
<i>Wistar</i>							
1	10.85 ± .93			60			Same
2	4.26 ± .68	1.47 ± .29		60	30		NaCl, NaHCO ₃ , H ₂ O
3	8.82		1.90 ± .36	30		30	NaCl, NaHCO ₃ , H ₂ O KCl, CaCl ₂ , MgCl ₂
4	5.63	2.99		30	30		Same
Grand Means							
<i>Sherman</i>	7.11	4.06	5.78				
<i>Wistar</i>	7.39	2.23	1.90				

NaHCO₃. Their intakes were recorded daily and individually for 10 days. Mean intakes and standard errors of the means were computed first for single rats and, hence, for units of 10 rat days. These results are shown in the unshaded bars of figure 6. The daily intakes were then added as if each group of 3 rats had been together in one cage; the corresponding results for the customary 30 rat days are shown by the shaded bars of figure 6, with standard errors of each mean shown to the right as a vertical line.

These single rats showed large differences in their partition of Na intake between HCO₃ and Cl. With respect to NaCl plus NaHCO₃, however, the results were more uniform. The four lowest intakes were found in the hypertensive rats (105, 83, 13, 85). One control rat had an intake less than that of 2 hypertensive rats but yet the

average intakes of NaCl, NaHCO₃ and the total of NaCl plus NaHCO₃, computed on the basis of 30 rat days, showed the usual significantly reduced intake of these salts by the hypertensive animals. Hence, it appears that in exploratory studies of the type used here it is justifiable, or at least expedient, to use groups of 3 rats to determine trends, but this does not exclude occasional departures from the mean behavior on the part of individual rats. This is not surprising in view of the well

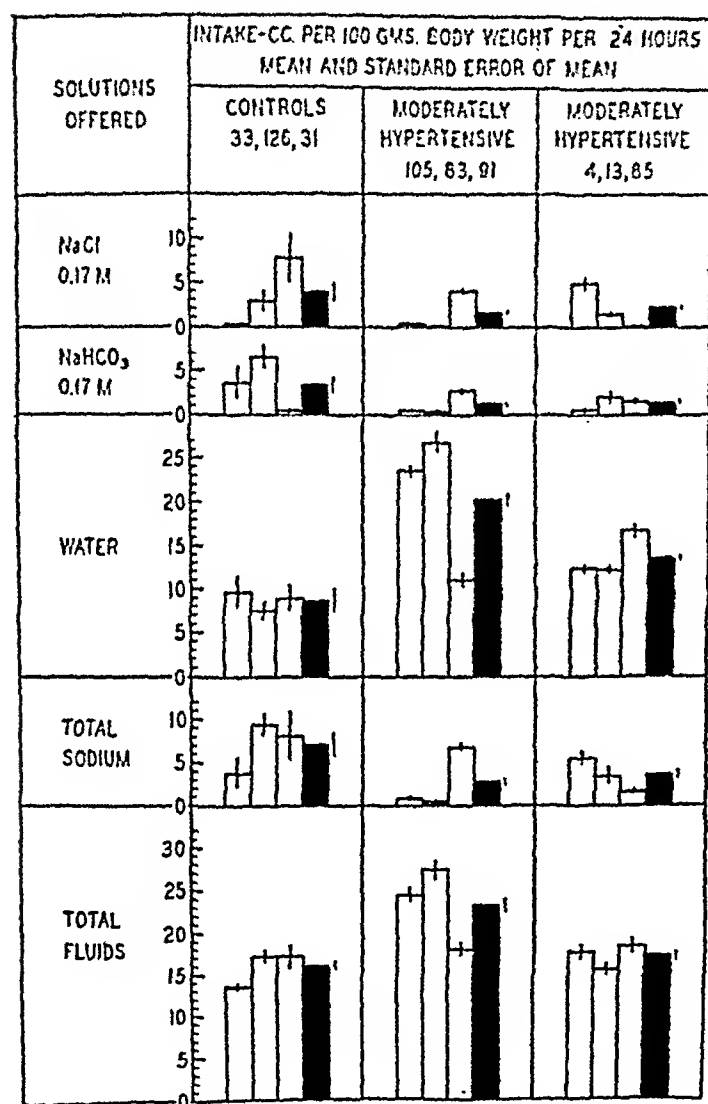


Fig. 6. CHART SHOWING intakes of water and 0.17 M. solutions of NaCl and NaHCO₃ by rats a) as individuals (open bars) and b) as groups of 3 (solid bars.)

known differences in the elective intake of individual rats even under control conditions (3, 4).

'Tasting' Error When Minute Amounts of Relatively Concentrated Solutions Must Be Selected. Other than NaCl, NaHCO₃, and possibly NaBr, very small volumes of salt solutions were taken, without detectable differences between normal and hypertensive animals. This might indicate a) that these minute quantities were selected equally and accurately or b) that the amounts were generally small and equal because of chance errors arising from random tasting of various solutions before finding water or the desired solutions of NaCl or NaHCO₃.

These two alternatives were tested by offering dilute or concentrated solutions of certain salts, together with water and 0.17 M. NaCl and NaHCO₃. One series of 3 normal and 3 hypertensive rats was offered 0.17 M. KCl, NaBr and MgCl₂, while 0.017 M. solutions of these three salts were offered to a second and similar series of animals. At the end of 15 days the solutions were reversed so that those rats previously offered 0.17 M. solutions were now offered 0.017 M. solutions and vice versa, for another period of 15 days.

The results were pooled for totals of 60 rat days with the clear result that the normal rats did not generally take 10 times as much of the weaker solutions. For 0.017 M. KCl, NaBr and MgCl₂, their intakes were respectively 1.6, 1.5 and 2.7 times those which they showed for 0.17 M. solutions of the same salts. Hypertensive rats took slightly more of the dilute solutions, viz. for 0.017 M. KCl, NaBr and MgCl₂ their intakes were respectively 9.6, 2.6 and 5.9 times that showed for 0.17 M.

TABLE 3. INTAKES OF 0.17 M. SOLUTIONS OF CERTAIN OTHER SALTS, TOXIC AND NON-TOXIC, BY NORMAL AND HYPERTENSIVE RATS

SALT SOLUTIONS 0.17 M.	INTAKE—CC/100 GM. BODY WT/24 HR.			
	Normal, 60 rat days	Hypertensive		
		Moderate	Severe	
			young, 30 rat days	old, 30 rat days
Lithium chloride.....	0.12 ± .02		0.12 ± .03	0.08 ± .02
Strontium dichloride.....	0.11 ± .03		0.05 ± .02	0.07 ± .03
Cadmium dichloride.....	0.10 ± .02		0.06 ± .03	0.07 ± .07
Boric acid.....	0.30 ± .08	0.10 ± .03	0.33 ± .01	0.25 ± .08
Manganese dichloride.....	0.08 ± .02	0.08 ± .03	0.19 ± .01	0.09 ± .04
Cobaltous chloride.....	0.25 ± .05	0.12 ± .04	0.17 ± .05	0.10 ± .04

solutions of the same salts. As usual the hypertensive animals took definitely less NaCl, NaHCO₃ and total Na solutions throughout. Therefore, when relatively concentrated solutions are taken in small amounts, it may be concluded that gross appetite for these substances is absent but it is still possible that small differences in self-selective intake could be shown to exist. Detection of such small differences would require offering solutions sufficiently dilute to reduce the 'tasting error' to a minimum.

Initial Studies on Selection of Certain 'Trace' Substances. The potential importance of minute traces of certain salts or organic compounds in maintaining normal function and growth is well established for animals and also for plants, as indicated by the recent monograph of Stiles (10). As an illustration of part of a systematic and general screening study, now in progress, comprising a wide variety of ions, table 3 summarizes results with six salts which fall in this 'trace' group. Some of these, particularly cobaltous chloride, are known to be toxic to animals, even in small amounts.

Two groups of normal and three of hypertensive rats were used. For one

period these rats were offered water and 0.17 M. solutions of NaCl, NaHCO₃, LiCl, SrCl₂ and CdCl₂, observing meanwhile effects on blood pressure, body weight and food intake. The intakes of the last three salts are shown in the upper half of table 3. The results of similar studies with 0.17 M. boric acid, MnCl₂ and CoCl₂ are shown in the lower half of table 3. In all instances intakes were small and essentially similar, their magnitude being in or below the range of the 'tasting error' mentioned above. Food intake did not change and body weight showed the usual slow rise throughout.

While the first three salts were offered, the average systolic blood pressures of the control rats fell by a slight but not significant amount, 6.8 per cent; while the blood pressures of the hypertensive animals fell even less, 0.4 and 2.4 per cent. When the second group of salts was offered, the blood pressures of the control rats rose by an apparently significant amount, 13 per cent, and those of the hypertensive animals rose slightly but not significantly, viz., 7 per cent for each group. These elevations developed shortly after the solutions were offered and were transient because all pressures returned to their prior levels within 30 days after the salts were withdrawn.

It can be concluded that hypertensive rats did not exhibit any gross appetite for these particular salts and that the amounts ingested neither increased nor decreased their hypertension significantly. Such minor rises of blood pressures as were obtained with the second group of salts in control rats may well have been due to early toxic effects because boric acid can produce renal damage (11) and cobalt produces a polycythemia (12) though toxicity may be reduced by concurrent administration of manganese (13). Nevertheless, 2 of the hypertensive rats died, one during and one shortly after these salts were offered. To determine whether one or more of these substances can elevate blood pressure of normal rats merits further and more detailed study.

DISCUSSION

The observation that hypertensive rats, given free choice, consistently elect to take less NaCl than normal rats do adds another item of evidence in favor of the apparent relation between certain forms of hypertension and the manner in which the body handles NaCl. This relation has been postulated *a*) because drastic restriction of sodium intake reduces the blood pressure of some hypertensive patients (14, 15) and of rats (9); *b*) because the administration of NaCl increases the hypertensive effects of desoxycorticosterone (16, 17); and *c*) less directly, because of the well known reduction of blood pressure associated with adrenal insufficiency as well as the rise of blood pressure sometimes found with hyperfunction of the adrenal gland.

Though moderately hypertensive rats often reduced their NaCl intake to less than half that of normal rats, their blood pressure remained unchanged. This agrees with the observation of Grollman and Harrison (9) that drastic restriction of sodium intake is required to produce a significant effect on hypertension. Grollman *et al.* (18) found, however, that a forced high NaCl intake by rats did not further increase the blood pressure of rats previously made hypertensive by partial nephrectomy as done by Chanutin and Ferris (19), nor does excessive NaCl per se produce or increase hypertension in man (20). This apparently is true also for the type of experimental hypertension produced by latex rubber capsules. When moderately hypertensive

rats were offered only 0.17 M. NaCl and NaHCO₃ without access to water, the summed intake of these two sodium solutions increased markedly and progressively reaching the very high levels of 27, 30 and 40 cc/100 gm. B.W./24 hrs. Blood pressure and body weight did not change significantly until shortly before death. The two rats taking the largest amounts of these sodium salts died on the 12th and 29th day of this regimen. Grollman *et al.* (18) described no fatalities but in their observations the high sodium chloride intake lasted only five days.

The mechanism responsible for the voluntary restriction of NaCl intake by experimentally hypertensive rats is at present unexplained as is also the earlier interesting observation by Barelare and Richter (21) that pregnant rats exhibit a markedly increased appetite for NaCl. Moreover, only one form of hypertension,—that produced by the perinephritis due to a latex capsule,—has been tested so far.

In these animals, however, the changes in NaCl intake appeared to be quite closely related to the development of hypertension. In the first place, preliminary studies in this laboratory by Sobin (22) indicated that the lessened appetite for NaCl appeared not immediately following the application of latex rubber capsules, but only after two or three weeks as blood pressure began to rise. Sham operated animals showed a very slight and insignificant reduction in NaCl intake which returned to normal. Second, in the present series, those few animals which did not develop systolic blood pressures of at least 165 mm. Hg after encapsulation sometimes maintained a normal appetite for NaCl or showed an insignificant decrease. Hence, it appears that mere encapsulation of the kidneys is not sufficient to change the appetite for NaCl but that a concurrent significant hypertension is also necessary. Third, in one experiment the 'control' groups included by error a few animals with blood pressures consistently in the range of 140 to 150 mm. Hg and these groups showed NaCl intakes below those of many other control groups from which all rats with systolic pressures of more than 135 mm. Hg had been carefully excluded by several preliminary measurements. It is possible that spontaneously developing hypertension in rats is also accompanied by lessened appetite for NaCl.

Since the changes in NaCl intake did not produce evident dehydration or edema, nor affect body weight, it may be inferred that the excretion and intake of NaCl were in approximate balance. Determinations of urinary chlorides in a few hypertensive and control rats were in agreement with this inference. The change of intake might therefore be due to a primary change of appetite per se, or to a change of appetite which was secondary to a change of excretion rate. If the latter is present, it might be due *a)* to disturbed adrenal function, *b)* to changed renal function by reason of the perinephric irritation produced by the capsule or *c)*, as a less likely mechanism, to a change in pituitary function. Studies to be reported in another paper (23), make it appear that the adrenal glands are not responsible for the diminished intake of NaCl by hypertensive rats. With respect to the possible rôle of the kidney, it is interesting that the NaCl intake of severely hypertensive rats, while less than normal, was still slightly greater than the intake of those with moderate hypertension. This may be related to greater loss of Na or Cl in the urine in severe hypertension because of greater renal damage. This point will be discussed in greater detail later (23).

Finally, it is noteworthy that hypertensive rats exhibited no special appetite

for certain ions which have been given orally for hypertension or arteriosclerosis, viz., I, NO_2 and SCN . It may be objected that these salts were given as the Na salts, but the striking partition of Na intake between chloride and bicarbonate, whenever the latter was available, and the equally striking neglect of Na citrate, suggest that had there been any special appetite for these ions, a similar partition of intake would have been found. At any rate, there was no striking modification of intake such as is observed for calcium and phosphate in parathyroidectomy or for NaCl in adrenalectomy (4).

Forced intake of NH_4Cl protects rats from the hypertensive and arteriosclerotic effects of desoxycorticosterone (16), but no evidence of voluntarily increased intake of NH_4Cl was observed here in perinephritic hypertension nor when desoxycorticosterone was given to normal and hypertensive rats (23). Similarly the first group of six trace elements did not contain any which affected hypertension or for which special appetite was exhibited.

The consistent average intakes of NaCl and NaHCO_3 , the consistent average differences in these intakes shown by normal and hypertensive rats and the equally consistent rejections of the other salts offered, suggest that this method may be profitably extended to those salts and foods not easily available in the ordinary diet of the laboratory rat. It is conceivable that reduction of hypertension, or the detection of special appetites, during such offerings may provide clues concerning the metabolic disorders which underlie hypertension in general.

SUMMARY

Rats made hypertensive by enclosing their kidneys in latex rubber capsules were offered water and 0.17 M. solutions of various salts to determine whether or not the hypertensive state was associated with any change of appetite for specific salts, and whether or not existing hypertension could be modified by self-selection of salts.

The mild polydipsia of hypertensive rats was confirmed. When offered 'free choice' of various solutions, including solutions of NaCl and NaHCO_3 , hypertensive rats elected to take only one third to one half as much NaCl and NaHCO_3 as did normal rats tested concurrently. This restriction of intake was more striking in moderately hypertensive rats than in those with severe hypertension, but was not complete enough to restore blood pressure to normal.

The intakes of Na citrate, Na_2SO_4 , Na_2HPO_4 , KCl, CaCl_2 and MgCl_2 were quite small and were not detectably different in normal and hypertensive animals. Minute differences in appetite for these salts cannot be excluded, however, due to a 'tasting error' when relatively concentrated solutions are offered.

Hypertensive rats showed no special appetite for certain salts which have been described as producing temporary alleviation of hypertension, viz. NaSCN , NaNO_2 , NaI and NH_4Cl . All these salts were taken equally and in small amounts by normal and hypertensive rats with no change of blood pressure. NaBr was taken in greater amount by a few moderately hypertensive rats with lowering of blood pressure secondary to torpor and reduced food intake, but the results were not consistent enough to be really significant.

Salts of certain 'trace' elements, viz., boron, manganese, cobalt, lithium, stron-

tium and cadmium were taken in small and approximately equal amounts by normal and hypertensive rats, without significant effects on blood pressure.

The consistent and significant reduction of NaCl and NaHCO₃ intake by hypertensive rats adds another item of evidence in favor of some as yet unexplained relation between the hypertensive state and sodium metabolism. The feasibility of using the self-selection technique in exploring possible metabolic disorders associated with hypertension is discussed.

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ANESTHESIA AND GASTRIC SECRETION¹

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DURING experiments carried out in this laboratory on anesthetized dogs with gastric fistulae it was noted that there was frequently a marked and unaccountable secretion of gastric juice. An investigation was therefore made of the influence of anesthesia itself on gastric secretion.

A combination of chloralose and urethane has long been an anesthetic favored for acute experiments on gastric secretion (1, 2). Chloralose anesthesia has also been widely used for this purpose (3-5). Most of our experiments were carried out with the chloralose-urethane combination which contains one part chloralose to 10 parts urethane by weight so that the animal receives one half the anesthetic dose of each drug. Chloralose, urethane, pentothal and nembutal were also tested individually.

It has always been assumed that there is little or no secretion of acid gastric juice under anesthesia unless specific subsequent measures are taken to produce it. Hence a vast number of acute experiments on anesthetized animals involving various procedures employed to analyze the gastric secretory mechanisms have been reported, e.g. electrical stimulation of the vagi and hypothalamus; administration of histamine, gastrin and other secretory stimulants; the retarding influence of atropine, entero-gastrone etc., on secretions elicited by various means; the influence of antihistamine compounds on histamine-evoked secretion; the supposed gastric secretagogue liberated following peripheral antidromic stimulation, etc. (e.g., 2-5). In view of this, the fact that chloralose and chloralose-urethane anesthesia were themselves found capable of eliciting a marked gastric secretion was felt to be methodologically important and possibly of theoretical significance.

METHODS

The experimental procedure involved anesthetizing a 24-hour fasted animal by intravenous administration of the drug. All dogs carried brass gastric cannulae inserted at least two weeks prior to the experiment. Immediately following anesthesia the animal was suspended in a canvas frame and juice collected from the fistula. The head was allowed to fall forward to insure external drainage of any saliva. Regurgitation of colored duodenal material was rare and if encountered the experiment was terminated. Subcutaneous saline was administered to replace fluid loss. A total of 35 such experiments was carried out on 12 dogs. To reduce psychic secretion just prior to anesthesia the animal was never fed in the experimenting room nor by the experimenter.

The pH of the juice was measured by the Beckman pH meter, peptic activity by the Nirenstein and Schiff modification of Mett's method described by Hawk (6), mucus by measuring the percentage of non-filterable material through no. 1 filter paper, and blood sugar levels by the micro method of Hagedorn and Jensen (7).

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RESULTS

A. Preliminary Observations

Stimulating action of anesthesia on gastric secretion. The number of experiments, volume, pH and peptic activity of the gastric juice with each drug is shown in table 1. The data are presented as an average for the large number of experiments with chloralose-urethane and individually for the others. Chloralose itself at times evoked a greater flow of gastric juice than did chloralose-urethane in the same animal but was not used as frequently since its insolubility necessitates administration of large amounts of fluid. However, the marked secretion under chloralose and our impres-

TABLE 1. SUMMARY OF DATA OF GASTRIC SECRETION WITH VARIOUS ANESTHETICS

ANESTHESIA	TESTS	AVERAGE SECRETION	pH	PEPSIN ¹
		cc/hr.		mill U/cc.
Chloralose-urethane	22	29.8 Average all expts.	0.88-1.0	0-4
Chloralose	4	1) 22.7 2) 53.3 3) 13.8 4) 45.8	0.90-1.05 0.88-0.90 1.0-1.1 0.99-1.02	0-50 290 0-4 0-4
Urethane	3	1) 29.1 2) 40.4 3) 8.5 ²	0.96-1.02 1.01-1.03 1.1	0 0-4
Pentothal	2	1) 25.3 2) 13.6	0.96-1.0 1.0-1.05	4-16
Nembutal	4	1) 0-14 2) 0-11.8 3) 0-4.5 4) 0	0.93 1.2-1.5 1.2-1.5	16

¹ The pH and pepsin ranges are those observed during active secretion following the washing-out process. ² Stopped in 20 minutes.

sion that secretion was more persistent than with chloralose-urethane indicate that its effect merits further investigation.

Despite the variation in volume with different (and to some extent with the same) anesthesia, highly acid secretion occurred in all but one experiment with nembutal. In all experiments with the latter there was a delay of approximately two hours before acid secretion occurred. A similar delayed response was observed in 4 of 22 experiments with chloralose-urethane. In general, secretion occurred in the first 20-minute collection.

In 8 of 22 experiments with chloralose-urethane the secretion was either much reduced or arrested after periods of secretion from one to three hours. In the other 14, active secretion was still occurring when the experiment was deliberately ter-

minated (or atropine given) after one to five hours of active secretion. In the 4 experiments with chloralose, secretion was still active after 1 hour, 3 hours 20 minutes, 3 hours 40 minutes, and 5 hours 40 minutes. In one experiment with urethane it was active after 1 hour 20 minutes and much reduced in the other two after 20 minutes and 1 hour 20 minutes respectively. With pentothal secretion was active at termination after 1 hour 40 minutes in one experiment and much reduced after 1 hour in the second. It will be noted that the figures for nembutal are given as 0-14 and 0-11.8 etc. in table 1. This is due to the fact that the secretion even when present with nembutal was erratic and came in sudden short-lasting spurts, in addition to having a long latent period and being of small volume. The second figure for nembutal represents the maximum secretion observed in an hour. Minor fluctuations in depth of anesthesia did not alter the secretory reaction but it tended to disappear.

TABLE 2. GASTRIC SECRETION DURING CHLORALOSE-URETHANE, PENTOTHAL AND URETHANE ANESTHESIA AND ITS ARREST BY ATROPINE

CHLORALOSE-URETHANE			PENTOTHAL			URETHANE		
Volume	pH	Pepsin	Volume	pH	Pepsin	Volume	pH	Pepsin
cc/20 min.		Melt U/cc.	cc/20 min.		Melt U/cc.	cc/20 min.		Melt U/cc.
3.2	1.85	170	11.4	1.02	36	6.5	1.01	42
14.6	1.16		11.3		16	6.4	0.95	19
10.5	1.05		5.0	0.92		10.3	1.2	4
25.7	1.01		7.2		4	12.6	0.96	0-4
26.5	0.98	16	7.3	0.98		7.8	1.02	0-4
23.0	0.95	0-4	1.5 mg. atropine			1.5 mg. atropine		
10.2	1.02		2.2	1.08	4	4.8	1.07	0
0.9			0.7			0.9		
0.0						0.4		

with either deep anesthesia or sub-anesthetic doses. It was our impression that the range of anesthesia with nembutal which resulted in acid secretion was very narrow, being restricted to a range of light anesthesia just greater than sub-anesthetic doses. The range of effective anesthesia appeared greatest with chloralose alone.

Since the animals were negatively conditioned to the experimental situation, acid secretion just prior to anesthesia was rare and the gastric mucosa was usually neutral or alkaline. On rare occasions when the animal was secreting actively prior to anesthesia, it was allowed to stand in the frame until secretion subsided. Secretion occurred after anesthesia, however, irrespective of the pre-existing state of the gastric mucosa. Hence it cannot be a simple perpetuation of a pre-anesthesia psychic secretion.

Composition of the juice. VOLUME. As seen in table 1 the average hourly secretion in a large number of experiments with chloralose-urethane was substantial. At times this combination, or chloralose alone, gave an hourly secretion of 80 cc. Protocols of separate experiments with chloralose-urethane, urethane and pentothal are shown in table 2.

THE pH. Following the initial 'washing-out' of mucus and food particles the pH of the juice almost always fell to 0.9 to 1.0 (table 2). After this it remained remarkably constant as long as secretion persisted. Frequently the pH values were in the range of the maximum values of histamine juice in dogs of $0.91 \pm .02$ as observed by Hollander and Cowgill (8). It would therefore appear that there was always a maximal activation of the acid secretion.

The secretion obtained with nembutal anesthesia, in addition to being by far the least and most erratic, did not regularly fall to as low a pH as did the others (table 1). This difference in acidity is probably due to greater neutralization by washed out or slowly secreted mucus with the small volumes of acid secretion.

PEPSIN. Although the pepsin values started at varying levels they rapidly fell to barely detectable amounts with chloralose-urethane. This was observed in 7 experiments with chloralose-urethane in which pepsin determinations were made. This characteristic of the secretions resembles the rapid fall and subsequent low peptic activity of histamine juice in dogs as observed by Babkin (9) and by Gilman and Cowgill (10).

In the small number of pepsin determinations made during active secretion with the other anesthetics (table 2), except in one instance with chloralose, the pepsin values of the juice were remarkably small. In this one experiment with chloralose, however, the pepsin values rose during secretion reaching approximately 300 Mett units/cc.

MUCUS. Following the initial washing out the secretion obtained with chloralose-urethane contained practically no visible mucus. On two occasions 60-cc. samples of chloralose-urethane juice were filtered through no. 1 filter paper. The non-filterable component constituted only 0.5 and 1 per cent of the total. This again is in accord with the reported low mucus content of histamine juice (1). This was also grossly true of the other anesthetics, except that considerable amounts of visible mucus were present in one experiment with chloralose mentioned above in which large amounts of pepsin were also detected.

Effect of atropine. Atropine sulfate in subcutaneous doses of 1.5 mg. and occasionally 2 mg. was administered in 4 experiments with chloralose-urethane, 2 with chloralose, 1 with urethane and 1 with pentothal. This dosage regularly either completely or almost completely abolished the secretion. In the latter instances a very slight residual secretion of acid juice persisted. This was always less than 1 cc/20 minutes. This sensitivity to atropine suggests an important rôle of the nervous system in the mechanism of the secretion. The effect of atropine is also shown in table 2.

B. Further Analysis of the Chloralose-Urethane Phenomenon

Effect of trauma on the secretion. In view of the fact that very many acute experiments on gastric secretion have been carried out (see Introduction), and this gastric secretory phenomenon of anesthesia never been reported we attempted to investigate possible explanations for it. Almost all previous experiments under anesthesia involved some relatively severe traumatic procedure such as insertion of the gastric cannula at the time of experiment. It had already been observed by Babkin,

Schachter and Nisse that additional trauma such as extirpation of the pylorus could arrest gastric secretion for as long as 2 hours despite continuous electrical stimulation of the vagus nerve (11). We therefore decided to see what effect trauma had on the chloralose-urethane secretion. Immediately following the insertion of the cannula the animal was suspended as before and secretion observed. Three such experiments were carried out with chloralose-urethane and the secretion subsequently observed for 1, 2½ and 3 hours, respectively. One was carried out with nembutal with a subsequent observation period of 2½ hours. In no instance was there the slightest acid secretion in the periods observed. Approximately 3 weeks later following complete recovery, all 4 animals secreted actively following chloralose-urethane anesthesia. Evidently the secretion had been completely inhibited by this operative procedure.

Effect of vagotomy. Two types of experiments were carried out to assess the rôle of the vagi in this secretion.

1. EFFECT OF ACUTE VAGOTOMY. Six experiments were carried out for this purpose. Following anesthesia with chloralose-urethane the cervical vagi were exposed as rapidly and gently as possible and threads loosely placed around the vagi which were then allowed to fall back into position. This procedure took approximately 7

TABLE 3. EFFECT OF ACUTE BILATERAL CERVICAL VAGOTOMY ON CHLORALOSE-URETHANE GASTRIC SECRETION

VOLUME ¹	7.5	5.0	12.2	11.3	12.8	10.6	10.3	Vagi cut	3.2	0.2	0.2
pH	1.32		1.08		1.0	0.96					
PEPSIN ²	70		16		0	0					

¹ cc/15 mins. ² Mett U/cc.

minutes. In 3 of these experiments the mere exposure and slight manipulation of the vagi resulted in the complete absence of any acid secretion for the entire period of observation, viz., 2 hours, 4 hours 10 minutes, and 4 hours 30 minutes, respectively. In one instance considerable secretion of an erratic nature occurred and in 2 experiments it was active and regular. In the 2 latter instances the cervical vagi were suddenly sectioned during secretion with the result that secretion was immediately arrested and remained so for the subsequent observation periods of several hours (table 3). The 3 animals whose secretion was completely arrested all secreted actively several days later when the vagi were not exposed.

2. EFFECTS OF CHRONIC VAGOTOMY. Three dogs previously found to secrete actively with chloralose-urethane anesthesia were aseptically vagotomized. One dog by subdiaphragmatic vagotomy, another by subdiaphragmatic section plus section of the left cervical vago-sympathetic trunk and a third survived bilateral cervical vago-sympathectomy for 3½ months. In the latter, section of all vagal fibers to the stomach is assured. All animals showed essentially the same reaction, viz., the restoration of the gastric secretion obtained with chloralose-urethane shortly following recovery from vagotomy and its subsequent almost complete disappearance as time was allowed for degeneration of the cut nerves to occur. Three weeks after the operation in all three instances the gastric secretory response to chloralose-urethane

was tremendously reduced and frequently unobtainable at all thereafter. The sequence of events is most clearly demonstrated by the dog with bilateral cervical vago-sympathectomy since the slight trauma incurred by this operation permitted an experiment with chloralose-urethane to be performed 3 days post-operatively. The secretion was not only restored but was greater than the pre-vagotomy secretion. Nevertheless it almost completely and permanently disappeared again after several weeks. Protocols of experiments with this dog are shown in table 4.

Another observation of interest in view of the controversy regarding variations in acidity of pure gastric juice (1) was frequently seen in the chronically vagotomized dogs first appearing about 4 weeks post-operatively. This consisted of the sudden liberation of fairly large amounts of a neutral or slightly acid, non mucoid, slightly

TABLE 4. EFFECT OF CHLORALOSE-URETHANE ANESTHESIA ON SECRETION OF SAME DOG BEFORE AND AFTER BILATERAL CERVICAL VAGOTOMY

MAY 28		MAY 31		JUNE 29		JULY 8	
Volume	pH	Volume	pH	Volume	pH	Volume	pH
cc/20 min.		cc/20 min.		cc/20 min.		cc/20 min.	
3.3		22.5	1.08	17.5	4.93	3.0	2.98
6.8		25.6	0.90	3.5	4.21	4.5	1.15
9.1	1.08	14.1		2.3	2.75	2.3	
11.0		7.2	0.94	7.5	1.5	0.6	1.65
8.0	1.02	9.6		0.8		0.8	
6.0		1.5 mgm. atrop.		0.4		0.9	
9.7				0.8			
Vagi cut		7.6	0.92				
		2.8					
2.4		0.9					
0.2							
0.2							

turbid (but not bile stained) fluid within the first few minutes following anesthesia with chloralose-urethane. At times more than 30 cc. of juice of pH 6 was obtained within 20 minutes following induction. On some occasions this neutral or slightly acidic juice began to flow rapidly following anesthesia in an animal whose mucosal reaction prior to anesthesia had been more acidic, which indicates the activation of a specific neutral secretion. The limited number of observations precludes any definite conclusions but is strongly suggestive that secretion of a non-mucoid, neutral juice by the gastric glands is possible under certain conditions. This phenomenon is evident in table 4.

Effect of chloralose-urethane on blood sugar. Since insulin and the amino acids exert a gastric secretory action by virtue of hypoglycemia (1) we observed blood sugar levels during active secretion with chloralose-urethane. In 2 experiments not only was there no fall in blood sugar after anesthesia but a slight rise of approximately 10 per cent. This is in accord with the slight hyperglycemia effected by the barbiturates and other anesthetics (12).

DISCUSSION

The degree to which moderate trauma may inhibit an abundant gastric secretion as evident from our experiments with chloralose-urethane, should be considered in interpreting experiments involving traumatic procedures. The mechanism whereby the chloralose-urethane secretion is prevented is nevertheless of interest. The fact that acute vagotomy completely arrests a secretion in progress but that this secretion returns to full intensity within a few days suggests that the arrest of secretion is due to some activity of the vagus nerve resulting in a peripheral refractory state of the secretory cells. There are two possibilities for the mechanism of such a phenomenon: *a*) efferent impulses passing down the vagi might render the neuro-cellular secretory mechanism resistant to further stimuli; *b*) arrested secretion could be secondary to constriction of the gastric blood vessels. The presence of efferent vaso-constrictor fibers in the vagus has never been demonstrated. However, the possibility of afferent impulses in the vagi reflexly causing vaso-spasm via the splanchnics cannot be ruled out. Pavlov long ago claimed the existence of 'secretory-inhibitory' fibers in the vagus supply to the stomach and pancreas (13). His concept however has since been opposed and has been neglected in physiological theories of the nervous control of secretion (14). Nevertheless, the complete degree of inhibition observed with acute vagotomy and at times even with slight manipulation of the vagi suggest that Pavlov's concept still merits consideration.

The consistent minimal amounts of mucus and pepsin plus the high acidity of the juice obtained with chloralose-urethane raised the question whether or not histamine liberation might be an effective link in the mechanism of this secretion. Babkin (15) and Emmelin and Kahlson (16) have suggested the possibility that histamine may play a physiological rôle in the nervous phase of gastric secretion. However the fact that insulin, amino acids (1), and ethyl 3:3 dimethyl allyl barbituric acid (17), which are believed to act through the vagi, all result in a juice containing considerable mucus and pepsin tended to contradict this theory. The fact, however, that chloralose-urethane secretion markedly resembles that of histamine yet is largely dependent on integrity of the vagi indirectly supports the possibility of the mediation of histamine in this phase of secretion.

We cannot offer a completely satisfactory explanation for the variation in secretion rate, onset and duration of secretion in the 22 experiments with chloralose-urethane, nor with the other drugs. Numerous factors such as depression of the blood pressure, circulatory inadequacy etc. might secondarily influence the secretory reaction. Further, since the gastric secretory response to insulin shows considerable quantitative variation (18), it is possible that these fluctuations are inherent in variations in sensitivity of the nervous mechanism.

SUMMARY

Chloralose and chloralose-urethane anesthesia cause a marked secretion of very acidic gastric juice in dogs previously equipped with gastric fistulae and not subjected to any trauma at the time of anesthesia. Urethane, pentothal and to a much lesser degree nembutal anesthesia also result in the secretion of varying amounts of gastric juice of high acidity under similar conditions.

The chloralose-urethane phenomenon was especially investigated. This secretion was regularly found to approach a pH of 0.9 and to possess minimal amounts of pepsin and visible mucus, thus resembling the secretion evoked by histamine. It was completely abolished by the traumatic procedure of preparing the gastric fistula at the time of the experiment, and was at times completely prevented by slight mechanical manipulation of the vagi in the neck. In those instances where the phenomenon was not abolished by exposure of the cervical vagi it was immediately arrested following acute vagal section. The secretion returned to full intensity within a few days after vagal section only to almost completely disappear again as time for nerve degeneration elapsed. The secretion was not associated with a reduced blood sugar level but was regularly abolished by atropine. It was markedly reduced or unobtainable in chronically vagotomized dogs.

The possible rôle of histamine in the nervous phase of gastric secretion and the possibility of a nervous mechanism inhibiting the secretory cell directly are discussed. Evidence is presented to suggest that the gastric glands may secrete a neutral or weakly acidic, non-mucoid juice under certain conditions.

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NONEXCRETION OF SERUM ALKALINE PHOSPHATASE BY THE LIVER AND THE PANCREAS OF NORMAL DOGS

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THERE are two theories concerning the origin of the alkaline phosphatase in the bile (1). One theory holds that the alkaline phosphatase of the blood serum is excreted into the bile (2); the other states that the alkaline phosphatase which appears in the bile is formed by the liver cells (3, 4). Freeman and Chen (5) have determined the rate of disappearance of abnormal concentrations of phosphatase from the circulation of normal dogs. The slow disappearance of the injected phosphatase was interpreted by them as indicating that if the liver excreted serum phosphatase into the bile, it did so only very slowly. It was considered desirable to obtain more direct evidence on excretion of serum alkaline phosphatase in the bile.

This study was designed to determine directly the phosphatase concentration of bile and its minute output in normal dogs in acute experiments before and after the transfusion of serum from a dog with obstructive jaundice. In order to get some information on the origin of pancreatic alkaline phosphatase, similar studies were conducted on the pancreatic juice simultaneously.

METHOD

These experiments were carried out on a chronic donor and acute recipient dogs. The donor was a large dog (weighing 20 kg.) whose common bile duct had been ligated 10 to 75 days prior to the time of transfusion. The recipients were all small-sized (5.5-7.5 kg.) dogs which were anesthetized with an intravenous injection of pentobarbital (32 mg/kg. body weight). Prolonged anesthesia was maintained when necessary by additional intravenous injections. The last feeding of the animals was 24 hours before the experiment. The trachea was cannulated and a cannula was also placed in the left femoral vein for secretin infusion. To preclude stimulation of the pancreas by acid in the duodenum the pylorus was occluded. The cystic duct was clamped. Bile and pancreatic juice were collected by cannulas inserted into the common bile duct and the major pancreatic duct respectively.

By means of a perfusion pump, highly purified secretin dissolved in saline was continuously injected intravenously at a moderate rate, averaging 0.83 mg/hr., into the left femoral vein of the animal. This secretin preparation was about 20 times as potent as SI (6). The purpose of using secretin was to stimulate the pancreas in order to collect enough pancreatic juice for analysis. Secretin also stimulates bile secretion, but in the doses used the effect was small. Both the bile and the pancreatic juice collected during the first hour of response to secretin was discarded (7).

Approximately 250 cc. of blood was collected from the donor by femoral artery puncture. It was centrifuged and the 100 to 120 cc. of serum so obtained was injected intravenously into the recipient animal. The injection was usually completed within 10 minutes. A small sample of serum from the donor was reserved for alkaline phosphatase determination. The bile and pancreatic juice were collected from the recipients in 40-minute portions throughout the whole experimental period, in graduated centrifuge tubes on ice, and was stored in the freezing unit of the refrigerator overnight. Three samples each of bile and pancreatic juice and one sample of blood were collected before the injection of serum. After the injection, blood samples were drawn from the recipients from time to time up to 3 to 5 hours. All samples were assayed for alkaline phosphatase content.

The method used for the estimation of alkaline phosphatase was essentially the one described by Shinowara, Jones and Reinhart (8). The estimation of the 'initial' inorganic phosphate and of the 'total' inorganic phosphate after incubation was devised by Dr. J. Canepa of this laboratory after Holman's method for inorganic phosphate. This method was outlined briefly in a previous communication (7). For bile samples, it is necessary to decolorize by using acid-washed activated charcoal before analyzing for inorganic phosphate (9).

From the alkaline phosphatase content of the various samples the number of units secreted per minute in the bile and pancreatic juice was calculated by multiplying the concentration expressed in U/100 cc. by the volume of the sample in cc. divided by 100 times the length of collection of the sample in minutes.

RESULTS

The concentration of the enzyme in the blood of the recipient dogs before and after the injection of the high phosphatase serum in the four experiments is shown in table 1. All 4 dogs showed a normal level of serum alkaline phosphatase before the injection. After the injection, the concentration of the enzyme in the blood was greatly increased in all. Even at the end of 5 hours the concentration was still maintained at a high level, although all showed a tendency to decrease slowly.

The volume, enzyme concentration and enzyme minute output of the bile and the pancreatic juice obtained before and after the injection of serum with high phosphatase content are shown in tables 2 and 3. The volume of the bile samples showed a tendency to decrease toward the end of the experiments while the concentration of phosphatase increased to such an extent that the enzyme minute output in the bile remained essentially unchanged. As can be seen from table 3, the volume of the pancreatic juice showed a tendency to increase after the injection of the serum. This was accompanied by a decrease in phosphatase concentration so that, just as with bile, the enzyme minute output in the pancreatic juice remained essentially unchanged or rather slightly decreased.

DISCUSSION

These results disprove the belief that the serum alkaline phosphatase is normally excreted in the bile or in the pancreatic juice. The alkaline phosphatase in the bile and in the pancreatic juice, therefore, is mainly formed by the liver and the pancreas respectively. In obstructive jaundice, owing to the fact that the normal

channel of escape of phosphatase is blocked, the alkaline phosphatase formed in the liver is released into the blood stream, thus causing the rise of the enzyme content in the serum. This is analogous to the rise of the serum amylase and lipase which accompanies obstruction of the pancreatic duct.

In the case of the pancreas, an elevation of serum alkaline phosphatase has also been reported to occur with obstruction of the duct (10). However, the liver injury

TABLE 1. SERUM ALKALINE PHOSPHATASE CONCENTRATION OF RECIPIENT DOGS BEFORE AND AFTER THE INJECTION OF HIGH PHOSPHATASE SERUM (4 DOGS)

DOG NO. AND SEX	BODY WT.	VOL. OF SERUM INJECTION	ALKALINE PHOSPHATASE CONCENTRATION (U/100 CC. SERUM)									
			Injected Serum	Minutes after injection								
				0	20	30	80	120	150	180	220	300
	kg.	cc.										
1 ♂	7.5	120	128	8.8	37.0		37.0	35.2	34.0	33.4		
2 ♂	6.5	100	182	2.8	41.0		36.2		35.4		32.9	31.9
3 ♂	5.5	120	132	5.8		101.7		76.9			34.7	41.4
4 ♀	6.0	120	140	2.8		70.2		55.8			51.8	48.0

TABLE 2. ALKALINE PHOSPHATASE CONCENTRATION AND MINUTE OUTPUT IN BILE BEFORE AND AFTER THE INJECTION OF HIGH PHOSPHATASE SERUM (4 DOGS)

	DOG	SAMPLE NUMBER ¹												
		1	2	3	Inject. of high phosphatase serum	4	5	6	7	8	9	10	11	
Volume (cc.)	1	2.8	2.6	2.4		2.2	1.8	1.5	1.0	0.8				
	2	2.8	2.9	2.9		3.7	3.2	2.8	2.6	2.5	2.1	1.8	1.9	
	3	1.4	1.3	1.3		2.3	1.5	1.0	0.9	0.6	0.6	0.5		
	4	2.0	2.0			1.6	1.6	1.5	1.5	1.6	1.6	1.5		
Alk. phosphatase concn. (U/100 cc.)	1	25.1	27.9	27.3		33.3	51.1	58.4	63.9	81.2				
	2	4.7	3.8	3.3		4.0	3.9	4.3	4.7	6.8	7.9	9.2	9.6	
	3	54.7	44.2	37.5		31.3	24.9	32.0	32.0	35.6	35.6	42.2		
	4	6.0	7.8			13.7	17.3	15.9	16.3	16.3	17.7	19.3		
Alk. phosphatase min. output (U × 10 ⁴)	1	176	181	164		183	230	219	160	162				
	2	33	28	24		37	31	30	31	43	42	41	46	
	3	192	144	122		180	93	80	72	53	53	53		
	4	30	39			55	69	60	61	65	71	72		

¹ All samples were collected in 40-min. portions.

which occurs after ligation of the pancreatic ducts is apparently responsible for the sustained elevation (9, 11).

While this paper was in manuscript Cantarow and Miller (12) reported on similar experiments carried out on duodenal fistula recipient dogs. Our data are generally in agreement with their results. It is of interest that they noted that the degree of elevation of serum phosphatase was greater than can be accounted for by the phosphatase activity of the injected serum. This indicates that a phenomenon of

activation is involved when normal serum and serum with elevated phosphatase are mixed (12-14).

In our experiments the rise in serum phosphatase in recipient *dogs 1* and *2* was approximately of the magnitude that would be anticipated on the basis of calculations using the phosphatase concentrations of the recipient and donor, plasma volume of the recipient and volume of the serum transfusion. By contrast, similar calculations for *dogs 3* and *4* indicate that considerable activation of phosphatase must have occurred upon mixing. It is particularly interesting to note that in these last two recipients, the phosphatase values fell rapidly over a period of several hours to the level that would be anticipated on the basis of calculations. This suggests that the 'activator' is rather quickly destroyed by the normal animal. The donor dog for all four experiments was the same animal. The blood transfused to the recipient

TABLE 3. ALKALINE PHOSPHATASE CONCENTRATION AND MINUTE OUTPUT IN PANCREATIC JUICE BEFORE AND AFTER THE INJECTION OF HIGH PHOSPHATASE SERUM (4 DOGS)

	DOG	SAMPLE NUMBER ¹											
		1	2	3	Inject. of high phosphatase serum	4	5	6	7	8	9	10	11
Volume (cc.)	1	11.6	12.8	14.2		14.7	16.0	17.5	16.0	15.1			
	2	3.2	3.2	4.7		8.3	8.0	9.1	9.7	9.8	8.9	7.9	9.3
	3	3.7	4.5	5.6		8.0	6.1	6.5	6.0	5.1	5.5	3.5	
	4	3.1	4.8	5.5		4.7	4.1	4.9	4.0	4.8	4.9	4.3	
Alk. phosphatase concn. (U/100 cc.)	1	1.8	1.8	1.6		1.2	1.4	1.4	1.0	1.4			
	2	12.0	10.8	8.4		6.2	6.3	5.5	5.3	4.8	5.4	5.6	5.2
	3	1.4	0.7	0.4		0	0	0.6	0	0.6	0.6	0.6	
	4	7.0	4.8	4.2		2.4	5.0	3.8	4.0	4.2	4.0	4.2	
Alk. phosphatase min. output (U × 10 ⁴)	1	52	58	57		44	56	61	40	53			
	2	96	86	99		130	126	125	129	118	120	111	121
	3	13	6	6		0	0	10	0	8	8	5	
	4	54	58	58		28	51	47	40	50	49	45	

¹ All samples were collected in 40-min. portions.

dogs 1 and *2* was drawn within one month after the biliary obstruction while that to the recipient *dogs 3* and *4* was drawn after 70 days. This might suggest that the 'activator' did not appear in the donor's serum until two months after the obstruction had been established.

SUMMARY

The increase in serum alkaline phosphatase caused by transfusion of serum from a dog with common bile duct obstruction to a normal dog does not result in an elevation of the output of alkaline phosphatase in the bile or pancreatic juice of the recipient. This finding indicates that the serum alkaline phosphatase is not excreted by the liver or pancreas and that the phosphatase which appears in the bile and pancreatic juice is formed by the secretory cells of these organs.

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BRADYKININ, A HYPOTENSIVE AND SMOOTH MUSCLE STIMULATING FACTOR RELEASED FROM PLASMA GLOBULIN BY SNAKE VENOMS AND BY TRYPSIN

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IN THE course of experiments on the physiological action of the venom of *Bothrops jararaca*, we found that some blood samples taken from a dog after the injection of minute doses of the venom had a stimulating effect upon the isolated gut of the guinea pig. This was not due to direct action of the venom since the gut had been previously made refractory to it; no desensitization could be observed after several additions of the serum to the perfusing bath containing the piece of guinea pig ileum. The stimulating principle disappeared from the blood very quickly after injection and could not be detected in samples taken a few minutes later.

Addition of the venom to defibrinated blood of a normal dog led to the release of a potent stimulating agent very similar to that detected in the blood after injection *in vivo*. That this substance could not be identified with any of the known pharmacologically active constituents of normal blood was made clear by preliminary tests and by experiments presented below. Among the well defined principles that have been described in the blood are: *a*) histamine, which is practically absent from dog plasma or serum (1); *b*) thrombocytin (2, 3) and/or SMC (4), two possibly identical principles extracted from platelets; *c*) kallikrein, a non-dialysable, heat labile, protein-like material, identified in normal plasma by Kraut, Frey and Werle (5, 6); *d*) the so-called 'früh-Gift' (7) that appears in an active form after the clotting of the blood and was identified by Zipf (8) as adenylic acid; *e*) acetylcholine, the presence of which in blood serum has been a matter of controversy (9-11).

The principle released in our experiments is dialysable through cellophane paper, is rapidly destroyed by the venom itself and by trypsin, and is not antagonized by antihistaminics and atropine. It could, therefore, be immediately distinguished from kallikrein, histamine, choline or acetylcholine. Since the principle appeared to be derived from plasma or serum, we were able immediately to rule out the possibility of its identity with thrombocytin or the SMC. Moreover, it was shown by Zucker (4) that SMC is resistant to digestion by trypsin, and we have found thrombocytin resistant to destruction by the venom of the *B. jararaca*.

The new factor, however, has certain analogies with the principle described by Feldberg and Kellaway (12, 13) under the name of *slowly reacting substance* (SRS). The latter was considered by Trethewie (14) to be released from rabbit spleen by snake venoms and trypsin. Although there is no indication that the new principle is identical with this slowly reacting substance, the contraction produced by it is of a slow type, starting after a short latent interval. We have, thus, given it the name *bradykinin*, indicating a principle which produces a slow movement of the gut. The globulin fraction (precipitated by a 30 to 45 per cent saturated solution of ammonium sulphate) from which bradykinin is released has been named *bradykininogen*.

MATERIAL AND METHODS

Defibrinated or oxalated ox blood was used in all large-scale experiments. In preliminary assays, we used dog blood and occasionally the blood of guinea pigs, rabbits and cats. Since red

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and white cells and platelets were found to contain little or none of the bradykinin precursor, they were discarded. Guinea pig and rabbit serums were used less often since they usually are strongly contaminated with histamine.

Preparation of Crude Bradykinin. Ox serum or oxalated plasma was treated with an equal volume of saturated ammonium sulphate at room temperature and left for a few hours. The precipitate, collected by centrifugation, was redissolved in two thirds of the original volume of distilled water and dialysed for 3 or 4 days against running tap water. The dialysed globulin solution (bradykininogen) was then neutralized. Aliquots of 200 cc. of the globulin solution were mixed with 30 cc. of a 0.1 per cent solution of the venom and incubated for 1 to 3 minutes at 37°C. The mixtures were then poured into two volumes of boiling ethyl alcohol, left for 5 minutes in a boiling water bath, and filtered. The filtrate was concentrated *in vacuo* and dried after washing with anhydrous ethyl ether and acetone. From each liter of the globulin solution, about 450 mg. of the buffy powder (crude bradykinin) was obtained. The activity of this powder is such that it usually produces a moderate contraction of the gut when 0.1 mg. is added to the bath. In terms of potency, the activity of the released material is almost completely recovered as 'crude bradykinin'.

A good stock of the venom of the *Bothrops jararaca* was used in a 0.1 per cent solution in distilled water for the large-scale preparation. Solutions of the venom for trial experiments were made in saline.

The venoms of *Naia naia* and *Denisonia superba* were kindly supplied by Dr. C. H. Kellaway, of the Wellcome Research Foundation, London. The venoms of *Bothrops atrox* and *Crotalus t. terrificus* were supplied by the Butantan Institute, São Paulo. The crystalline trypsin, containing about 50 per cent magnesium sulphate, was the commercial preparation of the Worthington Biochemical Laboratory, Freehold, New Jersey. The renin preparation and the angiotonin (hypertensin) were three-year-old preparations kindly supplied by Dr. I. H. Page, of Cleveland, Ohio. The renin, as tested, was in excellent condition and still fairly soluble. The angiotonin preparation was somewhat resinified with a brown, gummy appearance, but still strongly active upon the isolated gut and the blood pressure of the cat and the rabbit.

The determinations on the isolated gut were made in a Dale's apparatus, in the usual way, using a chamber of 7 cc. capacity.

RESULTS

Release of Bradykinin from Normal Blood by Snake Venoms and Trypsin. When the venoms of most species of snakes are put into contact with the uterus or the gut of the guinea pig, they cause a sharp increase of tonus that lasts for a long interval of time, even after repeated washings with new Tyrode solution. After relaxation, a second addition of the same dose of venom is either ineffective or produces a lesser effect, followed by a refractory state (desensitization) to any further addition of the venom. This effect, described by several workers (12, 13, 15, 16), was also described for trypsin (17) by one of us. If the guinea pig gut is made refractory to the action of snake venoms and trypsin, it can be used as a suitable test organ for the release of pharmacologically active substances from biological structures. On the other hand, the plasma of certain species (dog, ox and cat) is very poor in histamine and

therefore large doses of the plasma can be added to the perfusing bath without eliciting any response. When defibrinated blood or serum is added to the bath, we can occasionally detect the presence of minute amounts of an unidentified principle. The presence of this principle in normal serum is, however, erratic, as pointed out by Reid (2, 3) and confirmed in our experiments. When, however, a few micrograms of the venom of the *Bothrops jararaca* is added to a few cc. of blood or serum, a powerful contraction occurs after a short incubation of the serum with the venom. The maximum effect can be observed when the incubation is restricted to 1 to 3 minutes. If incubation is prolonged for 20 or 30 minutes, the active principle (bradykinin) disappears; this indicates that it has been destroyed by the further action of the venom (fig. 1). Crystalline trypsin has the same activity (fig. 2), although doses at least 5 times as great are necessary to produce an effect of similar magnitude. We can follow the generation of this substance by simply adding successively to the bath containing the gut 0.2 cc. of defibrinated blood or serum and 0.1 mg. of the venom of the *B. jararaca*. After a latency of 25 to 30 seconds the contraction of the gut sets in and proceeds at an increasing rate during the first 1 or 2 minutes (fig. 3).

We have been able to exclude entirely the blood elements as precursors of this stimulating principle. First of all, oxalated or heparinized plasma shows the same effect as whole blood, but even in a more striking manner. If the cells, after removal of the plasma, are resuspended in saline they show a much lesser effect than whole blood or cell-free plasma. Secondly, we could localize in a definite fraction of the plasma or serum proteins the precursor (bradykininogen) of this active principle. The total potency of the blood appeared to be concentrated in the globulin fraction, precipitated by a 30 to 45 per cent saturated solution of ammonium sulphate. As shown in table 1, the supernatants, after precipitation of plasma proteins with increasing concentrations of ammonium sulphate, showed less and less effect. When the concentration reached the 45 per cent level, no activity was found in the supernatant, while the full activity could be recovered from the redissolved and dialysed precipitate.

The first attempts to isolate bradykinin were very laborious due to the fact that the same agent that released it, after incubation for a few minutes, destroyed it if incubation was prolonged for a few minutes more (15-30 minutes). Both activities, that of release and that of destruction, appeared to be enzymatic, since they depended upon temperature and the actual concentration of the agent used. Release and destruction of bradykinin could be studied quantitatively (fig. 4). If 0.1 mg. of the venom of the *B. jararaca* was added to each cc. of blood or serum, the peak was observed after 3 minutes incubation, at 37°. If 0.3 mg. was added, the peak was somewhat earlier and destruction was complete after 10 minutes incubation. After complete destruction of the released bradykinin, further addition of the venom had no effect, the precursor being apparently exhausted. If, however, a small dose of the venom is added, say 20 micrograms, to each cc. of a fresh sample of the globulin fraction, a sort of equilibrium is attained in the reacting mixture, and even after one hour incubation, a small quantity of bradykinin can be detected in the system. If now, a larger dose of the venom is added, the release of considerable quantities

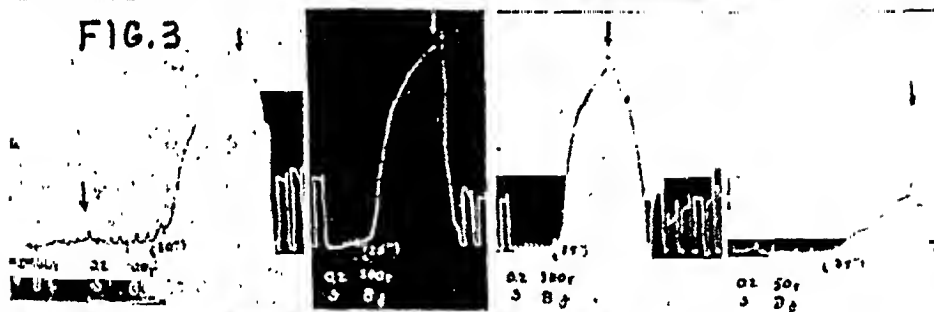
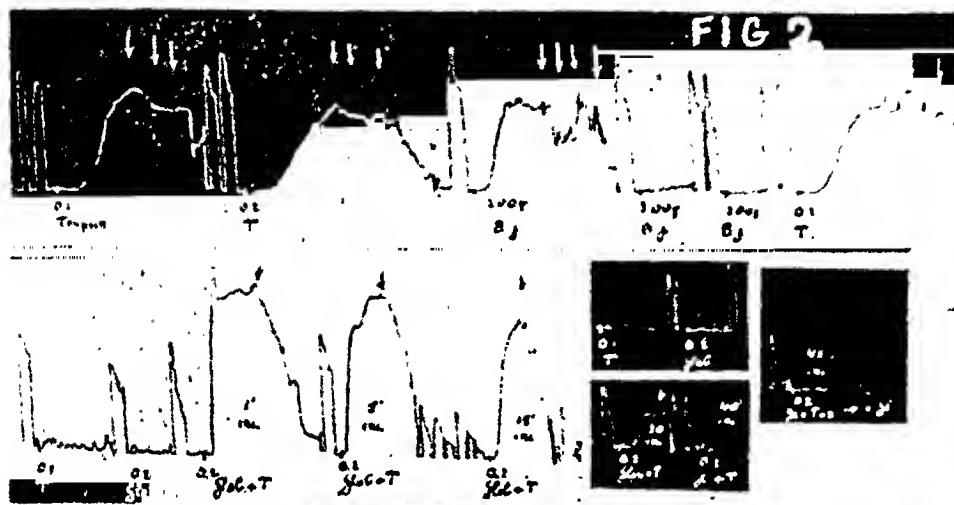
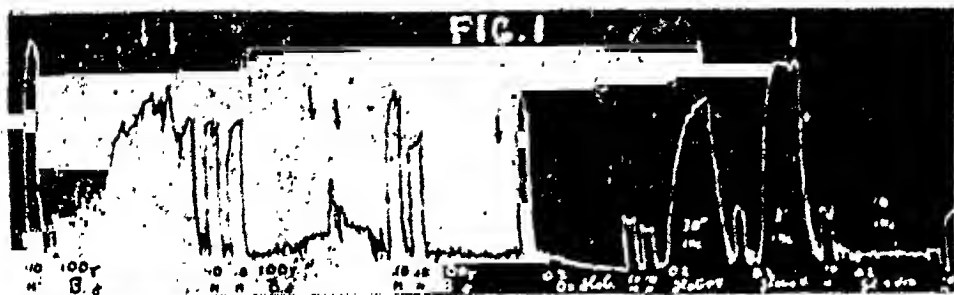


Fig. 1. GUINEA PIG ILEUM. After desensitization to 100 μ g. of the venom of *B. jararaca* (B.j.), the gut responds to the mixture of the globulin and venom (Glob. + ven.) incubated for 30" and 3 min. Further incubation of the mixture for 10 min. destroyed the stimulating principle. H = histamine 1:2 million.

Fig. 2. GUINEA PIG ILEUM. Upper tracing: The gut desensitized to venom still reacted with trypsin. Lower tracing: after the gut became refractory to 0.1 cc. trypsin, it reacted powerfully with a mixture of the globulin + trypsin, incubated for 1 min. After incubation for 45 min. the stimulating principle was completely destroyed; then a new fresh addition of the globulin was added with venom and a new contraction set in. T = 2.5 mg. crystalline trypsin/cc.

Fig. 3. GUINEA PIG ILEUM desensitized to the venom of *B. jararaca*; 0.2 cc. of ox serum was added to the bath, followed by addition of different doses of venom. The latency after addition of venom is indicated in parentheses. Maximum effect was obtained with 200 μ g. of venom. The shorter contractions were elicited with 0.2 cc. of histamine, 1:2 million.

Fig. 4. GUINEA PIG ILEUM desensitized to the venom of *B. jararaca*. A, incubation of ox defibrinated serum (for 1 and 4 min.) with 1/10 of its volume with a solution of 0.1% of venom releases activity, although 10 or 30 min. incubation entirely destroyed it; B, a smaller concentration of venom (1/50) released less activity, but destruction was also slower; C, a higher concentration of venom (1/3) released the maximum activity after 1 min. incubation, but destruction was rapid. After destruction of the principle, a new fresh mixture of serum + venom elicited maximal contraction.

of the active principle takes place; this shows that the precursor had not been exhausted by the action of the small dose of the venom.

If a large enough dose of trypsin is added to a fresh sample of the globulin, bradykinin is set free and exhaustion can be observed after a while. The venom of *Denisonia superba* and that of *Naia naia* were much less potent than that of the *Bothrops jararaca* in releasing bradykinin. We could not attain a concentration sufficient for exhaustion of the globulin when the latter was tested with the venom

TABLE 1. CONDITIONS OF EXTRACTION OF BRADYKININ, FROM OX SERUM OR GLOBULIN

Alcohol extraction:		Time of incubation:		
	ACTIVITY		ACTIVITY	
	%		% of max.	
(I) 5 cc. serum + 0.5 mg. venom, incub. 3 min.	100	(VIII) Mixture I incubated for:		
(II) Mixture (I) + 2 vol. alcohol; 5 min. boil.; filtrate conc. <i>in vacuo</i>	100		0'	10
(III) Mixture (I) + 1 cc. N HCl + 2 vol. alcohol; 5 min. boil.; filtrate conc. <i>in vacuo</i>	50		1'	60
			2'	80
(IV) Mixture (I) + 1 cc. 0.5 N NaOH + 2 vol. alcohol; 5 min. boil.; filtrate conc. <i>in vacuo</i>	100		3'	100
			4'	50
			10'	15
			30'	0
Thermo resistance:		(IX) Precip. with amm. sulphate:		
	ACTIVITY	Ox serum + amm. sulph.		ACTIVITY
	%	(% of saturat. room temp.)		Ppt. Supernt.
(V) Extract (II) + 0.2N NaOH heated for 10 min. at 90°	15	35	50	50
		40	70	30
(VI) Extract (II) + 0.2N HCl heated for 25 min. at 90°	100	45	100	0
		50	100	0
(VII) Extract (II) + 5 cc. concentr. HCl, heated for 1 hour at 90°	0			

Experiments III and IV show that extraction by alcohol is more efficient from the alkaline mixture, than from the acid one, although destruction of the isolated principle is more rapid in alkali (V) than in an acid medium (VI).

of the *B. jararaca* or trypsin. Crotoxin, a purified lecithinase of the venom of the *Crotalus t. terrificus* prepared according to Slotta *et al.* (18), did not release bradykinin nor did a purified preparation of lysolecithin. Therefore, we are bound to assume that the release of bradykinin is unrelated to the hemolytic activity of the venoms studied, but probably dependent upon their proteolytic activity. The capacity of the different agents studied are roughly parallel to their proteolytic and coagulant activity (table 2).

Biological Assay of Bradykinin. Until a pure preparation of bradykinin is available, we must use a temporary standard for comparison of activity. Since the

sensitivity of the gut to histamine or acetylcholine does not necessarily run parallel with that toward bradykinin, we abandoned the idea of comparing the strength of the unknown solutions of bradykinin with a histamine or acetylcholine standard. Moreover, the response of the gut toward these agents is quite different from the response to bradykinin. The latter is definitely slower in its onset and the increase in tonus follows a different course. Therefore, we decided to choose an early homogenized sample of crude bradykinin as a standard. This sample, called *Brady I*, has been used for comparison in all assays of purification referred to in this paper. For the purpose of comparing two different samples, the assay upon the guinea pig

TABLE 2. CORRELATION BETWEEN COAGULATING AND PROTEOLYTIC ACTIVITY OF VENOMS AND THEIR CAPACITY FOR LIBERATING BRADYKININ

AGENTS:	PROTEOLYTIC ACTIVITY		COAGULATING ACTIVITY ³		LIBERATION OF BRADYKININ ⁴
	Casein ¹	Globulin ²	Dose/cc.	Clotting time	
	mg. NPN/100 cc.		mg.	sec.	% max. act.
<i>B. jararaca</i>	13.8 (1 h)	0.89 (1 h)	1	36	100
	39.6 (24 h)	0.89 (1 h)			
<i>Trypsin</i>	37.7 (1 h)	0.83 (1 h)	7	91	30
	110.8 (24 h)	0.83 (1 h)			
<i>B. atrox</i>	14.7 (1 h)		1	45	30
<i>Naia naia</i>	5.6 (24 h)	0 (1 h)	1	no clot	traces
<i>Den. superba</i>	9.0 (24 h)	0 (1 h)	1	"	"
<i>Crotoxin</i>	0 (1 h)	0 (1 h)	1	"	0
<i>Lysocitlin</i>	0	0			0

¹ 2.5 cc. of a 2% casein solution + 2 cc. phosphate buffer (pH 7.4) + 0.5 of solution of venom or trypsin (1 to 2 mg./1 cc.); 1 or 24 hours incubation at 37° and the NPN estimated.

² 2.5 cc. of a globulin solution (bradykininogen) + 2 cc. phosphate buffer (pH 7.4) + 0.5 cc. solution of trypsin or venom (2 mg./1 cc.), 1 hour incubation at 37° and NPN estimated.

³ Coagulating activity was estimated upon citrated ox plasma: each tube containing 0.2 cc. of plasma + 0.2 cc. of the venom or enzyme solution (concentration indicated).

⁴ Activity in releasing bradykinin is indicated in reference to the potency of the venom of the *B. jararaca* (100%), using ox globulin as precursor.

ileum has been most useful. The response of the gut is fairly quantitative (fig. 5), allowing a satisfactory comparison between the unknown and the standard.

Quantitative estimations of the bradykinin precursor (bradykininogen) can be made by determining the maximal potency released by a fixed amount of the venom (e.g., 150 µg/cc. of serum or globulin solution) after incubation with the precursor for increasing intervals of time. It is obvious that this estimation has only comparative value since we cannot be sure that the whole stock of the active material has been released; on the other hand, if the concentration of the venom is increased beyond a certain limit destruction of bradykinin will be very rapid and the maximum peak will not be attained. In consequence, we must fix conditions for a routine comparison of potency of different batches of the globulin preparation. This procedure has been very useful in the large-scale preparations, in which samples of a few cc. of the globulin to be used were submitted to a standard dose of the venom,

for $\frac{1}{2}$ min., 1 min., 2 and 3 mins. incubation and the time for the maximal effect determined.

Purified Bradykinin. Although experiments are still proceeding in an attempt to further purify bradykinin, we have attained a second step in the procedure of purification that can be outlined now. 'Crude bradykinin' is redissolved in distilled water, filtered through a medium porosity sintered glass funnel, and 10 volumes of absolute ethyl alcohol are added with stirring. A flocculent precipitate is formed and removed without any loss of potency. The precipitate contains some inert organic nitrogen and gives a strong reaction with ninhydrin. Bradykinin, however, is fairly soluble in 80 to 90 per cent ethyl alcohol. The filtrate is dried *in vacuo*, and care is taken to avoid bubbling of air through it. A convenient way to do this is to use a separatory funnel connected with a Kitasato flask to which a strong vacuum pump is connected. The solution contained in the separatory funnel is allowed to fall dropwise to the bottom of the flask; this permits very quick drying in a moderately heated water bath. The material obtained in this way is about 8 to 12 times as active as the original 'crude bradykinin' and will be referred to in this paper as purified bradykinin (fig. 6). A highly potent material can be obtained, although in small yield, if the purified bradykinin is redissolved in 85 per cent ethyl alcohol and more absolute alcohol added until a faint precipitate is formed. The mixture left in the ice-box overnight shows a flocculent snow-white precipitate that may be 30 to 50 times as active as the crude preparation. This preparation has been obtained only in very small amounts.

Some Physical and Chemical Characteristics of Bradykinin. The active principle is very stable to heat, and resists prolonged boiling in distilled water or dilute HCl (boiling for 1 hour in a 0.1 to 1.0N HCl solution did not significantly decrease its potency). Boiling with a few cc. of concentrated HCl, as used in the method of Barsoum and Gaddum, as modified by Code (19), destroys its activity. Boiling for a few minutes in a 0.1N NaOH solution is enough to destroy bradykinin. It is a dialysable substance, insoluble in ether and anhydrous acetone. When absolute ethyl alcohol is used for the extraction of bradykinin, a large quantity of the active material is left behind. As the preparation becomes more purified it does not resist the manipulations to which we can submit the crude material. Especially, bubbling of air through the capillary becomes a very serious cause of loss of potency. This loss of potency by bubbling of air was found to be more important after precipitation of the inert material with 80 to 90 per cent ethyl alcohol, thus suggesting that, through this procedure, a stabilizer is removed.

All bradykinin preparations obtained up to now give a strong ninhydrin reaction and contain considerable organic nitrogen. Since trypsin liberates and destroys bradykinin, it is probable that release of bradykinin is the consequence of the rupture of a peptide linkage and that bradykinin itself is a polypeptide or, at least, contains a peptide linkage, the integrity of which is indispensable to its pharmacological behavior.

Pharmacology of Bradykinin. Bradykinin has been found to stimulate all smooth muscle structures so far assayed: the intestine and uterus of the guinea pig, the uterus and intestine of the rat, and the intestine of the rabbit. In our experi-

ments, the guinea pig ileum was the most sensitive, while the intestine of the rat was least so. The purified preparations of bradykinin (P. B.) elicited a strong response of the guinea pig gut, when added to the perfusing bath in doses as low as 2 to 10 micrograms; this corresponds to concentrations in the bath of 1:3 to 1:1 million, approximately. Since there are no indications that we are dealing with highly purified preparations, it appears probable that bradykinin is a very potent

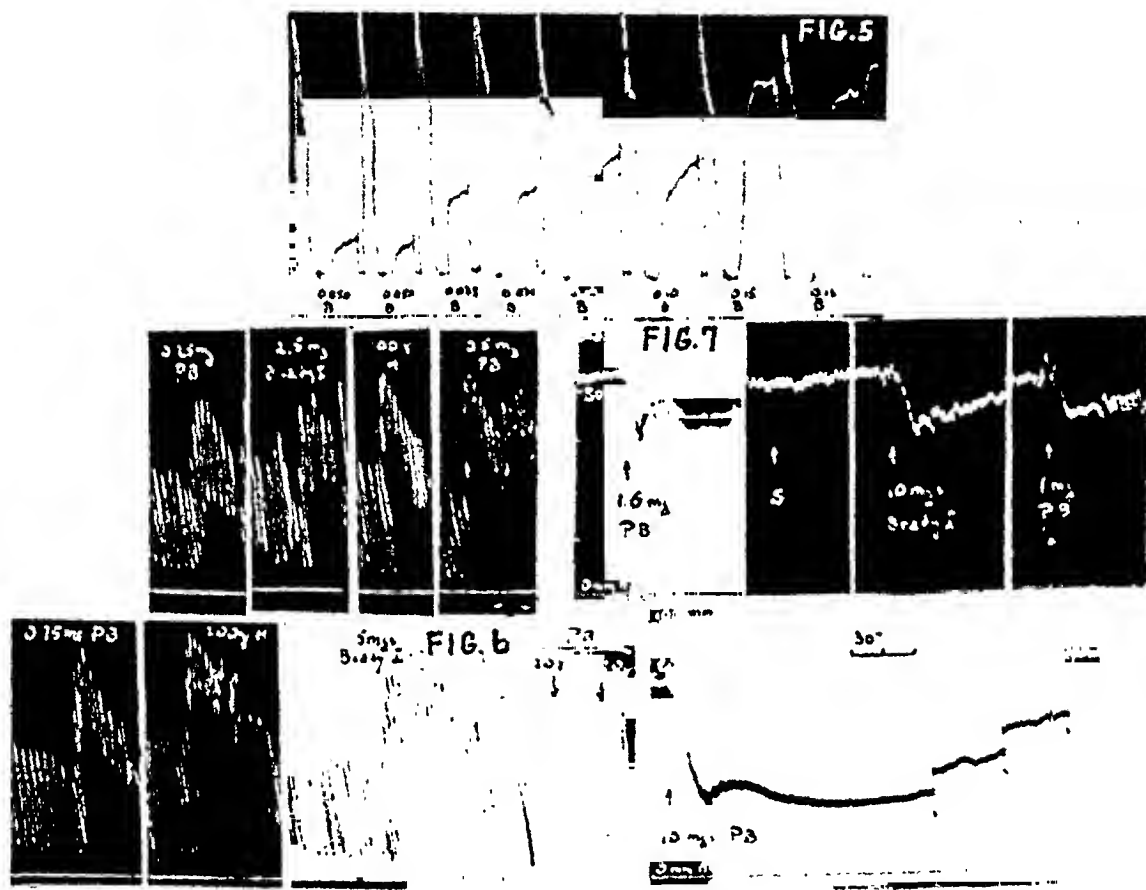


Fig. 5. GUINEA PIG ILEUM. Successive additions of increasing doses of a solution of bradykinin (B) produce graded responses that can be used for the bio-assay of bradykinin. The quick responses are due to histamine (0.2 cc. of a 1:2 million solution).

Fig. 6. RABBIT GUT. Effect of two different preparations of bradykinin. *Brady I* is a crude preparation, while *P.B.* is a purified bradykinin, about 10 times more active than *Brady I*. *H* = histamine hydrochloride.

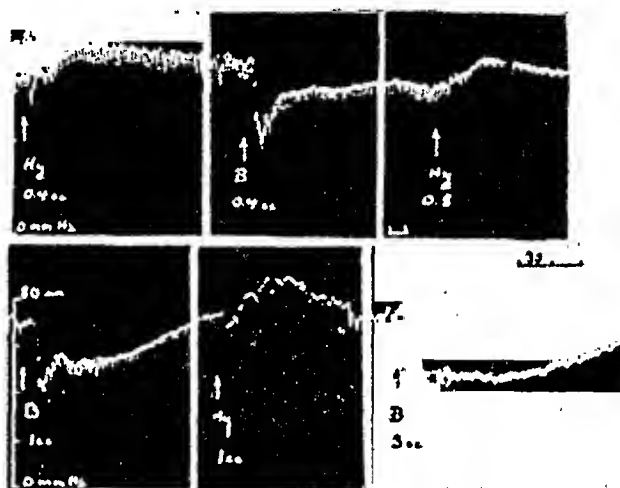
Fig. 7. CAROTID BLOOD PRESSURE IN THE RABBIT. Comparison between crude bradykinin (*Brady I*) and a purified preparation (*P.B.*) about 10 times as active as *Brady I*. Same preparations used in experiments of fig. 6. At \times the drum was stopped for 2 min.

pharmacological agent. When 10 or more mg. of the crude bradykinin (*Brady I*) is injected into the veins of rabbits and cats a sharp fall in arterial blood pressure follows. 'Purified bradykinin', when injected in a dose of 5 to 10 mg., produces a shock-like condition, with a steady fall in arterial blood pressure, followed by a very slow return to the initial level (fig. 7).

That the same principle (bradykinin) is involved in the effects upon both the smooth muscle and the arterial blood pressure is indicated by the fact that the same relationship of potency (1/10) was observed when crude (Brady I) and purified bradykinin (P. B.) were assayed for both actions (figs. 6 and 7).

A few quantitative considerations might help to evaluate the possibility of a release of bradykinin to explain the fall in blood pressure, when the venom or trypsin is injected intravenously. Ten to twenty micrograms of the purified preparation elicit responses in the guinea pig gut that are comparable to those elicited by 0.1 to 0.2 cc. of serum or plasma treated with an optimum amount of the venom. Therefore, 100 to 200 cc. of blood when incubated with the venom for a half minute to 3 minutes would release 10 to 20 mg. of 'purified bradykinin'. Since this amount is sufficient to produce a profound shock when injected intravenously, it becomes quite evident that there is enough available material in the circulating blood to account

Fig. 8. CAROTID BLOOD PRESSURE IN THE RABBIT. The same ox globulin preparation was treated with renin and with the venom of *B. jararaca*. The dried alcohol extracts were tested upon the rabbit. Hy = renin-treated preparation; B = the same substrate with venom.



for most of the hypotensive effect produced by the venom or by trypsin, when given to a normal animal.

Release of Bradykinin by Renin. It has been reported (20) that hypertensin (or angiotonin) contracts almost all smooth muscle structures, producing an effect that is strikingly similar to those described above. It has also been shown that renin when put into contact with the pseudo-globulin fraction of normal plasma or serum (hypertensinogen) releases a smooth muscle stimulating agent that has never been chemically separated from hypertensin (21). The following experiments suggest the possibility that this smooth muscle stimulating agent released by renin can be distinguished from hypertensin, and is probably identical with bradykinin.

We could confirm the facts described by previous workers that renin when incubated for 3 to 10 minutes with the globulin fraction releases a factor that contracts the guinea pig ileum. If incubation is prolonged for 60 to 90 minutes, the stimulating factor released by renin is entirely destroyed. If now, the same globulin preparation is treated with the venom, no further release takes place, showing that the bradykinin precursor has been exhausted. Conversely, if the globulin fraction is treated with an appropriate dose of the venom or trypsin, until it is exhausted of its

supply of bradykinin, it no longer releases any smooth muscle stimulating principle, if put into contact with renin.

Differentiation of bradykinin from hypertensin is very easy, since the material obtained from serum globulin by contact with the venom or trypsin has an effect upon the blood pressure of the cat or the rabbit opposite to that obtained by contact of the same material with renin. On no occasion could we observe any hypertensive effect of preparations obtained by treating bradykininogen (or hypertensinogen) with the venom of the *B. jararaca* or trypsin. These facts are clearly shown in figure 8.

Since renin appears to release bradykinin from the pseudo-globulin fraction of the blood, it seems logical to open the question of the identity of hypertensin and the smooth muscle stimulating principle generated by renin when incubated with the globulin preparation. The presumption that a hypotensive, myotropic agent as bradykinin is a common contaminant of the hypertensin preparations available would be analogous to the case of the oxytocic and pressor principles of the posterior hypophysis. These also have a polypeptide nature and are extremely difficult to separate by the usual chemical procedures, although by using more refined methods, evidence has been obtained that they constitute two separate principles (22).

DISCUSSION

Very minute doses of the venom of the *B. jararaca* (0.1 mg/kg.) render the blood incoagulable, when injected intravenously in a dog. After recovery from the mild shock so produced, the injection of a larger dose of the venom (0.5 mg/kg.) causes the animal to develop a severe shock that may lead to death. A quite similar situation is found in the so-called trypsin shock. After the injection of a small dose of crystalline trypsin, the blood becomes incoagulable; if however the dose is again injected, the animal develops a shock-like condition that is irreversible in many instances. The injection of a suitable dose of heparin previous to the injection of trypsin does not prevent death, but may even aggravate the development of the shock. Therefore, intravascular coagulation cannot explain death, but could be responsible for only a minor part of the symptoms produced by these agents. Liberation of histamine, previously thought to constitute an important factor in the production of trypsin shock, can only partially explain its mechanism. The amounts of histamine appearing in the circulating blood are small and by no means enough to explain the severity of the shock. After injection of the venom of *B. jararaca* we have frequently found a drop in blood histamine. There is no question that histamine is released from certain organs, by perfusion with trypsin (23) or the venom of the *B. jararaca*, but the amounts liberated are not of such magnitude as to produce a significant increase in the circulating blood. Moreover, it was shown by Wells *et al.* (24) that benadryl does not prevent trypsin shock in the dog. Although antihistamine substances are rather poor in preventing hypotension due to histamine itself, a significant drop in mortality of dogs treated with benadryl + trypsin should be expected, if the toxicity of the enzyme depended mainly on a liberation of histamine. All these facts would encourage one in looking for a new agent released from blood or tissues in poisoning by trypsin and certain snake venoms.

The presence of a powerful hypotensive and smooth muscle stimulating agent in the pseudo-globulin fraction of normal plasma or serum offers a new possibility for explaining the etiology of many symptoms occurring in the shock states produced by venoms and trypsin. This agent (bradykinin) is present in an inactive form (bradykininogen) and can be released by many agents besides the venom of the *B. jararaca*. Trypsin releases it and there is a certain correlation between the proteolytic and coagulating activity of snake venoms and their capacity for liberating bradykinin. The venoms of *Naia naia* and *Denisonia superba* that show slight proteolytic activity release very small amounts of bradykinin.

Experiments indicate that part of the bradykinin is released when blood clots, since there occurs a definite decrease in the bradykininogen potency when measured before and after the coagulation of the blood. This appears to indicate activation of the proteolytic system of the blood during the process of coagulation, as suggested by Ferguson (25). In connection with these findings it appeared important to study the behavior of platelets when disintegrated in contact with the globulin fraction. In some experiments, it appeared quite clear that platelets contain a factor that is able to release bradykinin from the globulin fraction. The experiments were complicated by the fact that platelets themselves contain another factor (SMC or thrombocytin) that contracts smooth muscle and produces a fall in arterial blood pressure. This factor could be definitely distinguished from bradykinin, since as shown by Zucker (4) it is resistant to boiling for one hour with 1.0N NaOH, a concentration of alkali that completely destroys bradykinin in a few minutes. Moreover, bradykinin is rapidly destroyed by incubation with trypsin or the venom of the *B. jararaca* whereas SMC or thrombocytin is entirely insensitive to these treatments.

There are many other questions that should be re-studied on the basis of the present findings. In some preliminary experiments, we have shown that the fibrinolytic enzyme activated by treating the serum with chloroform releases a gut-stimulating principle when incubated with the bradykininogen. Since activation of fibrinolysin occurs in many conditions of shock (26, 27), it is possible that release of bradykinin might explain much about shock of varying etiology. Furthermore, the origin and nature of the so-called anaphylatoxin of Friedberger and Bordet (28) has been an open question. Experiments by Dale and Kellaway (29) have shown that a smooth-muscle-stimulating principle is generated in normal guinea pig serum, by contact with starch or bacterial suspensions. At the same time, activation of proteolytic and fibrinolytic activity was shown in similar conditions (30). Activation of the proteolytic and fibrinolytic power of the blood during anaphylactic shock has been demonstrated (26) and, recently, Ungar (31) has also shown activation of a fibrinolytic enzyme when organs of a guinea pig are put into contact with peptone and the antigen. All these findings point to a 'proteolytic crisis' occurring in these kinds of shock and it is quite conceivable that release of bradykinin constitutes an aggravating factor in many conditions.

SUMMARY

The pseudo-globulin fraction (precipitated by 30 to 45% saturation with ammonium sulphate) of normal plasma contains the precursor (bradykininogen) of

a hypotensive and smooth muscle stimulating factor (bradykinin) that can be released by proteolytic and coagulating venoms of the *Bothrops* genus and by trypsin. If an appropriate dose of the enzyme or the venom is used, the maximum release is observed after 1 to 3 minutes incubation, at 37°. If incubation of the globulin with the venom of *B. jararaca* or trypsin is prolonged for 10 to 20 minutes the released factor is destroyed; this suggests that it has a polypeptide nature or at least a peptide linkage, the integrity of which is necessary for its pharmacological activity.

Bradykinin is thermostable, dialysable through cellophane, resistant to prolonged boiling in 0.1 to 1.0N HCl solution, but rapidly destroyed if heated in an alkaline solution.

The guinea pig gut is the most sensitive smooth muscle organ assayed and the rat intestine the least so. Doses of bradykinin equivalent to those released from 100 to 200 cc. of plasma produce a steady fall in arterial blood pressure of the cat, the rabbit and the dog. The possibility that bradykinin is a mediator in several kinds of shock is discussed.

We are indebted to Dr. Sylvia O. Andrade for the estimation of the proteolytic and coagulating activity of the venoms used. All experiments were done with the technical assistance of Mr. Jayme Ferraz. The venom of the *B. jararaca* used was the stock venom for preparing immune-serums, at the Butantan Institute.

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EFFECT OF HYPOPHYSECTOMY ON NEUROMUSCULAR FUNCTION

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AFTER hypophysectomy the magnitude of contraction of striated muscle during repetitive direct stimulation (1-3) decreases only to a moderate degree, whereas the spontaneous activity (2, 4, 5) is nearly abolished. The decrease of activity of striated muscle as well as the greater decrease of spontaneous activity suggest that both nerve and muscle are affected by the hormones of the pituitary gland.

The purpose of this investigation was to ascertain whether the function of nerve-muscle preparations of hypophysectomized animals is impaired and, if so, whether this dysfunction is due to a failure of the muscle or of both the muscle and the nerve.

METHOD

Hypophysectomy was performed on two strains of rats: on one strain by the Hormone Assay Laboratory, Incorporated, Chicago, and on the other by the authors. The latter strain was bred by the Department of Anatomy, Cornell University Medical College, New York. The hypophysectomy was performed either on litter mates or on rats from successive litters. The 33 hypophysectomized rats used were of the following sex and age: males: 5, one month old; 4, three months old; 4, six months old and 4 one year old; females 2, one month old; 4, three months old, 5, six months old and 5, one year old. The completeness of removal of the pituitary gland was established by autopsy. Rats from the strain of the Department of Anatomy, Cornell University Medical College, New York, served as controls and were either fasted for three days or were operated by a technic similar to that used on the experimental animals, except that the pituitary gland was not removed.

Indirect Stimulation. Electromyograms were taken during stimulation of the sciatic nerve by the following method: the spinal cord of the rats was destroyed up to the midthoracic region. The two legs were used as separate preparations. One leg was firmly fixed at the knee and ankle joints to an animal board. One recording needle electrode was placed into the upper half of the gastrocnemius muscle; the other, into the tendon of the gastrocnemius muscle. Stimulating needle electrodes were placed either near or directly on the sciatic nerve. The nerve was stimulated for periods ranging from a few seconds to three minutes with a stimulus having a repetition rate of from 3 to 30 pulses/second and of 'supramaximal' intensity. Stimulation at various frequencies was repeated a number of times for each preparation.

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The sweep circuit of the oscilloscope was synchronized with the stimulator so that successive stimuli and muscle action potentials were superimposed on the screen of the cathode ray tube. Either single or superimposed action potentials were photographically recorded from the screen of the cathode ray tube.

The area of the action potentials was measured with a planimeter; the amplitude, with a ruler. The values obtained at the beginning of a series of stimuli served as 100 per cent. The values obtained from the action potentials, taken either during or at the end of a prolonged stimulation, were expressed as a percentage of the values obtained at the beginning of each series of stimuli. All values deviating from 100 per cent by more than twice the square root of the sum of the squares of the standard error of mean of the controls and the standard error of mean of the experiments were considered as significant effects ($2\sqrt{S.E.^2(\text{controls}) + S.E.^2(\text{expts.})}$).

Direct Stimulation. One gastrocnemius muscle of 10 hypophysectomized rats was stimulated directly and indirectly. Stimulating needle electrodes were placed on both the sciatic nerve and the upper half of the gastrocnemius muscle. The muscle was stimulated with a stimulus having a repetition rate of 5 pulses/sec. and of 'supramaximal' intensity. This direct stimulation was delivered before and immediately after the interruption of the indirect stimulation with 11 pulses/sec. for 30 seconds. The muscle contraction was recorded by a kymograph through an isotonic lever attached to the end of the tendon of the gastrocnemius muscle.

Blood Circulation. Upon conclusion of the experiments, Berlin Blue was injected into the abdominal aorta of the rats to ascertain whether or not the blood supply of the gastrocnemius muscle remained adequate during the experiments. Each leg was uniformly discolored within a few seconds suggesting that the blood supply was adequate.

RESULTS

Indirect Stimulation. The first stimulus applied to the sciatic nerve was followed by the largest muscle action potential. In the control animals (table 1, fig. 1 A-D), the amplitude and the area of the action potential were unaltered at the end of 30 seconds stimulation with a stimulus of 3 and 5 pulses/second; the area and amplitude of the action potential had decreased on the average of 10 per cent by the end of 30 seconds stimulation with a stimulus of 11 pulses/second and on the average by 25 per cent by the end of 30 seconds stimulation with 30 pulses/second.

In the hypophysectomized rats (table 1, fig. 2 A-D), the amplitude and area of the action potential had decreased on the average by 17 per cent at the end of 30 seconds stimulation with a stimulus of 3 pulses/second; by 28 per cent with a stimulus of 5 pulses/second; by 55 per cent with a stimulus of 11 pulses/second, and by 85 per cent with a stimulus of 30 pulses/second. Recovery always occurred but it was slower in hypophysectomized rats than in the controls.

There was no significant difference between the behavior of the males and females, between rats belonging to the various age groups (one month to one year), and between rats of the two strains used. The decrease of the action potential during indirect stimulation in the hypophysectomized rats as compared to the control rats is statistically significant.

Direct Stimulation. Because of the number of previous investigations dealing with muscle contraction during repetitive direct stimulation in hypophysectomized animals, extensive studies of muscle contraction were not made in this study. It was, however, ascertained whether the decrease of muscle contraction at the end of 30 seconds indirect stimulation was maintained if adequate stimulation reached the muscle through direct stimulation.

In the control rats (fig. 1 *E*) the contraction of the gastrocnemius muscle remained unchanged during repetitive indirect stimulation (11 pulses/sec. for 30 sec.). The magnitude of muscle contraction on repetitive direct stimulation before and after indirect stimulation was the same.

In hypophysectomized rats (fig. 2 *E*) the contraction of the gastrocnemius muscle during repetitive indirect stimulation (11 pulses/sec. for 30 sec.) decreased on the

TABLE 1. EFFECT OF HYPOPHYSECTOMY ON THE ELECTROMYOGRAM

RATS		FREQUENCY OF STIMULATION (PER SEC.)										NO. OF EXPTS.	
Treatment	No.	3	5	11						30			
		Time of record after beginning of stimulation (sec.)											
		30	30	5	15	30	60	180	5	15	30		
		<i>Maximal amplitude of action potential (in % of the 1st action potential of each series)</i>											
Hypo- physec- tomy	33	82 ± 0.4	72 ± 0.9	83 ± 0.6	65 ± 1.1	47 ± 1.4	30 ± 1.8	18 ± 2.0	69 ± 1.0	34 ± 1.5	17 ± 2.3	221	
Fasted non oper.	10	98 ± 0.2	96 ± 0.4	98 ± 0.3	93 ± 0.5	90 ± 1.0	85 ± 1.1	80 ± 1.5	95 ± 0.2	80 ± 0.9	75 ± 2.0	30	
Sham- operated	10	100 ± 0.3	97 ± 0.6	99 ± 0.5	95 ± 0.7	91 ± 0.6	86 ± 1.2	82 ± 1.4	96 ± 0.4	82 ± 1.2	73 ± 1.8	28	
		<i>Maximal area of action potential (in % of the 1st action potential of each series)</i>											
Hypo- physec- tomy	33	84 ± 0.9	73 ± 1.3	86 ± 1.1	68 ± 1.5	44 ± 1.5	35 ± 1.6	21 ± 2.2	71 ± 0.9	39 ± 1.7	14 ± 2.8	221	
Fasted non oper.	10	97 ± 0.5	95 ± 0.7	97 ± 0.4	92 ± 0.6	89 ± 1.3	84 ± 0.9	81 ± 1.4	96 ± 0.5	79 ± 1.2	74 ± 1.6	30	
Sham- operated	10	99 ± 0.2	97 ± 0.4	98 ± 0.6	94 ± 0.9	92 ± 0.7	87 ± 1.0	81 ± 1.3	95 ± 0.6	83 ± 0.9	72 ± 2.1	28	

average by 50 per cent. (The action potential decreased on the average by 53 per cent.) The magnitude of muscle contraction on repetitive direct stimulation before and after indirect stimulation was approximately the same.

DISCUSSION

The above experiments show that in hypophysectomized rats the number of muscle fibers that contract during repetitive indirect stimulation decreased at a time when nearly all muscle fibers were capable of contraction provided impulses reached them. The decreased neuromuscular function was due mainly to a dysfunction of either the nerve or the myoneural junction since: 1) the magnitude of muscle contraction on direct stimulation before and after indirect stimulation was about the same; 2) the muscle action potential and magnitude of muscle contraction during

indirect stimulation decreased about 50 per cent; and 3) the muscle action potential on indirect stimulation after repetitive indirect stimulation recovered only gradually.

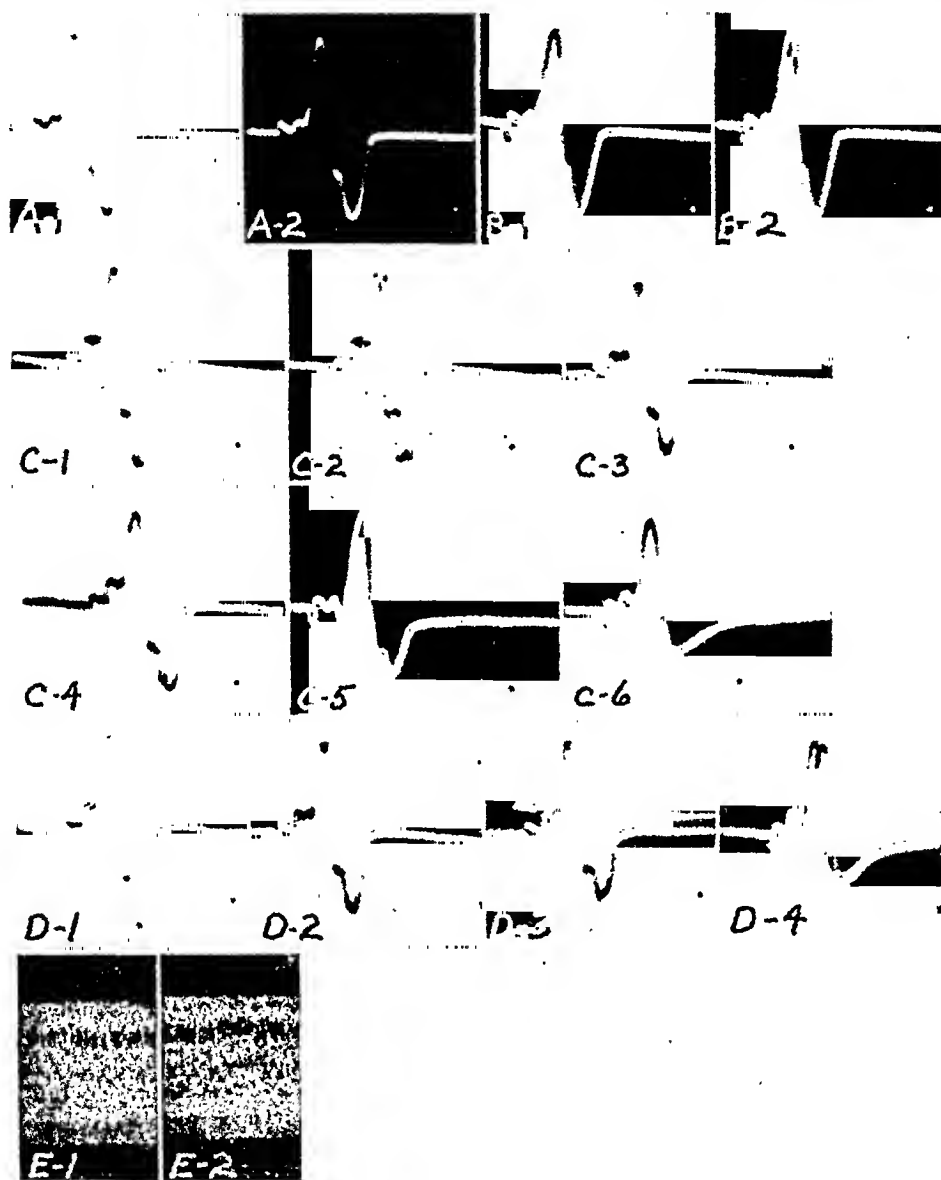


Fig. 1. ELECTROMYOGRAMS OF A CONTROL RAT (indirect stimulation). A. Stimulation with 3 pulses/sec. for 30 sec. 1. Record taken at the beginning of stimulation. 2. Record taken at the end of a 30-sec. stimulation period. B. Stimulation with 5 pulses/sec. for 30 sec. 1. Record taken at the beginning of stimulation. 2. Record taken at the end of a 30-sec. stimulation period. C. Stimulation with 11 pulses/sec. for 3 min. 1. Record taken at the beginning of stimulation. 2. Record taken at 5 sec. 3. Record taken at 15 sec. 4. Record taken at 30 sec. 5. Record taken at 60 sec. 6. Record taken at 180 sec. D. Stimulation with 30 pulses/sec. for 30 sec. 1. Record taken at the beginning of stimulation. 2. Record taken at 5 sec. 3. Record taken at 15 sec. 4. Record taken at 30 sec. E. Kymograph records of a control rat (direct stimulation). 1. Stimulation with 5 pulses/sec. immediately before indirect stimulation. 2. Same immediately after indirect stimulation.

The neuromuscular fatigue of hypophysectomized rats is independent of strain, age and sex. Also, it is not due to postoperative shock and weight loss due to anorexia of the rats, since operated control rats and emaciated starved rats did not show the decreased neuromuscular function.

Prolonged impairment of blood circulation due to hypophysectomy could not be prevented but sudden impairment of blood circulation did not enter as a factor in neuromuscular dysfunction observed, since the dysfunction occurred to the same

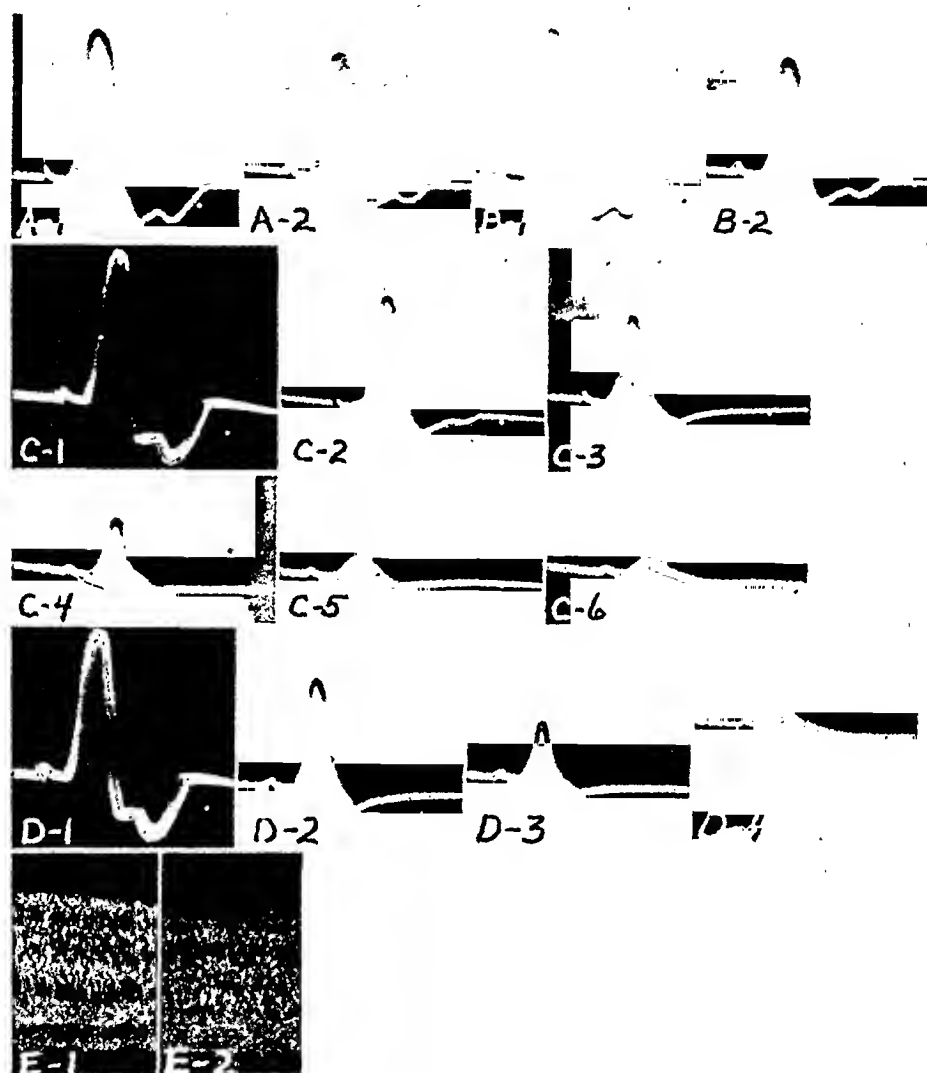


Fig. 2. ELECTROMYOGRAMS OF A HYPOPHYSECTOMIZED RAT (indirect stimulation). *A*. Stimulation with 3 pulses/sec. for 30 sec. 1. Record taken at the beginning of stimulation. 2. Record taken at the end of a 30-sec. stimulation period. *B*. Stimulation with 5 pulses/sec. for 30 sec. 1. Record taken at the beginning of stimulation. 2. Record taken at the end of a 30-sec. stimulation period. *C*. Stimulation with 11 pulses/sec. for 3 min. 1. Record taken at the beginning of stimulation. 2. Record taken at 5 sec. 3. Record taken at 15 sec. 4. Record taken at 30 sec. 5. Record taken at 60 sec. 6. Record taken at 180 sec. *D*. Stimulation with 30 pulses/sec. for 30 sec. 1. Record taken at the beginning of stimulation. 2. Record taken at 5 sec. 3. Record taken at 15 sec. 4. Record taken at 30 sec. *E*. Kymograph records of an hypophysectomized rat (direct stimulation). 1. Stimulation with 5 pulses/sec. immediately before indirect stimulation. 2. Same immediately after indirect stimulation.

degree in fresh preparations and in preparations after a few hours of experimentation. Also, injection of Berlin Blue at the end of the experiments showed an adequate blood supply.

Although the mechanism for the production of the neuromuscular dysfunction observed in hypophysectomized animals is not yet identified, it may be suggested that an impairment of acetylcholine synthesis occurs after hypophysectomy. This view is supported by the fact that administration of the adrenocorticotrophic hormone of the pituitary gland increases acetylcholine synthesis (6). Furthermore, measures that decrease acetylcholine synthesis also decrease neuromuscular function (7) and the action potential of nerve (8).

The present experiments suggest that the pituitary gland is necessary to maintain an optimal neuromuscular function by modifying the activity of both the nerve and the muscle.

SUMMARY

The effect of hypophysectomy on the neuromuscular function of rats was investigated. Hypophysectomy caused a decline of the amplitude and area of muscle action potential during repetitive indirect stimulation. This decrease occurred with relatively low frequency stimulation and was even more evident with increased frequency of stimulation. On indirect stimulation the decrease of muscle action potential paralleled the decrease of the magnitude of contraction. The magnitude of muscle contraction on direct stimulation decreased less than on indirect stimulation. It is inferred that the neuromuscular fatigue occurring in hypophysectomized animals is due to both a moderate dysfunction of the muscle and a marked dysfunction of the nerve element (either nerve or myoneural junction).

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COMPARATIVE STUDY OF ANTAGONISTIC DRUGS ON NORMAL AND DENERVATED EFFECTORS

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A DIRECT proportionality has been suggested to exist between the dose of acetylcholine necessary to stimulate an effector and the dose of atropine required to produce inhibition (1-3). An inverse relationship would apply to acetylcholine and curare. It was believed of interest to study this problem on effectors with an altered threshold for acetylcholine produced by chronic denervation and to compare the results with those obtained on the same normal effectors. Two types of effectors were used; one sensitive to muscarine (nictitating membrane, *n.m.*) and the other sensitive to nicotine (sympathetic ganglion, *s.g.*).

The experimental results enable us to discuss some aspects of the law of denervation as formulated by Cannon (4).

METHODS AND RESULTS

In all the experiments cats were anesthetized intraperitoneally with 1 ml/kg. of a 25 per cent urethane (Merck) solution, containing 3.3 per cent of sodium pentobarbital (Abbott's Nembutal). Immediately before the experiment and depending on whether the *s.g.* or the *n.m.* was to be studied, either the preganglionic fibers were sectioned or the superior cervical ganglion was extirpated on the normal side. The same operation was performed previously (4-13 days) under ether anesthesia on the opposite side. All cats had their adrenals removed.

The *n.m.* was stimulated with acetylcholine or adrenaline, depending on the drug under study. The stimulation of the *s.g.* was obtained with acetylcholine. In each effector the threshold dose and the dose producing a maximum response were determined. In actual experiments, a dose higher than the threshold dose was used; obtaining, therefore, a submaximum response.

Atropine at a dose of 1 mg/kg. of body weight, intravenously administered, was used in studying the *s.g.*, in order to block the direct response of the *n.m.* to acetylcholine.

The drugs used were atropine (Mallinckrodt), prostigmine (Roche), acetylcholine (Roche), ergotamine (Sandoz), dehydroergotamine (Sandoz), adrenaline (Clin), intocostin (Squibb), raw curare from Brazil, tetraethylammonium bromide (Sanitas) and β -erythroidine obtained from Professor Hug (Rosario, Argentina).

A. Nictitating Membrane. ATROPINE. In these experiments the *n.m.* was stimulated by simultaneous injections of acetylcholine in both carotids. Atropine

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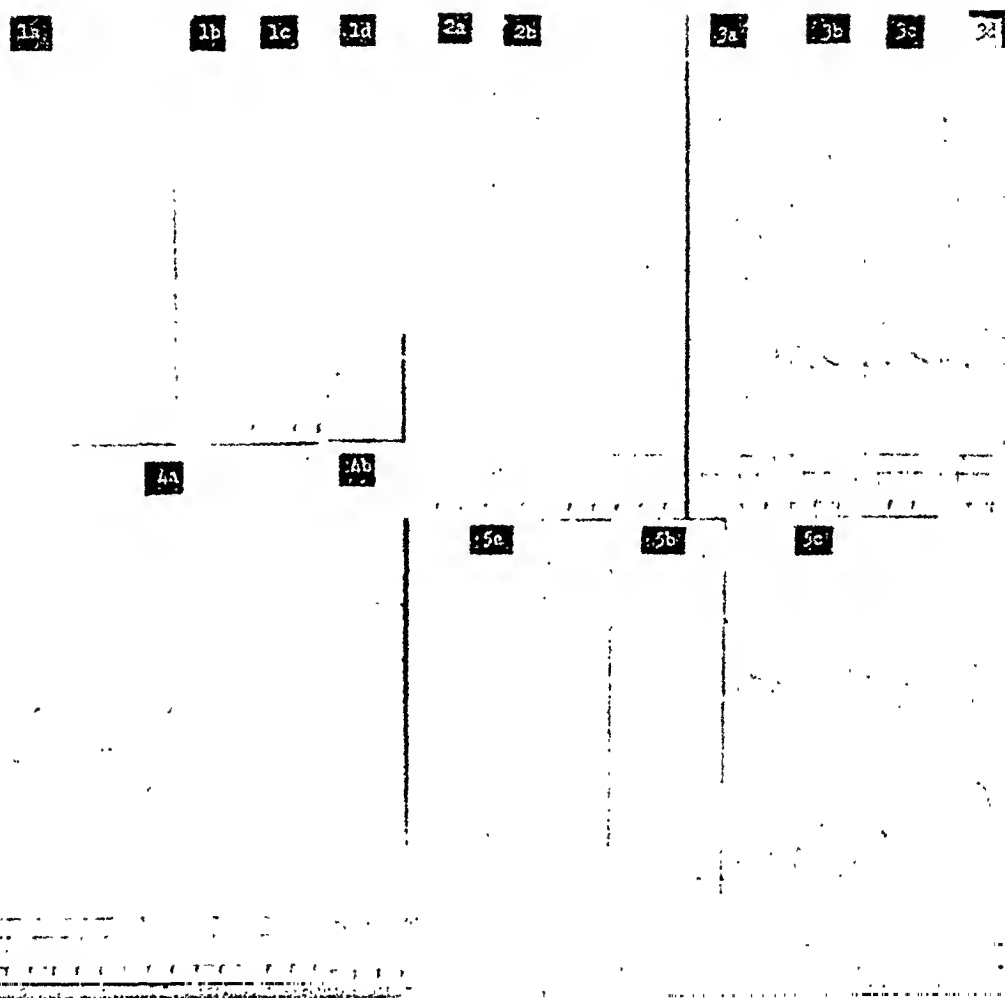


Fig. 1. EFFECT OF ATROPINE ON THE NICITATING MEMBRANE. *Above: n.m.* with a 6-day-old denervation. *Below: n.m.* control. Marks in upper line: 1 μ g. of atropine in both sides. Marks on middle line: acetylcholine 0.5 μ g. in denervated side and 5 μ g. in control. Marks on lower line: time in min. Between *a* and *b*: 8 min.; between *b* and *c*: 7 min.; between *c* and *d*: 45 min.

Fig. 2. EFFECT OF PROSTIGMINE ON THE NICITATING MEMBRANE. *Above: n.m.* with a 4-day-old denervation. *Below: n.m.* control. Mark on upper line: 2 μ g. of prostigmine in both sides. Marks on middle line: acetylcholine, 0.31 μ g. in denervated side and 2.5 μ g. in control. Lower marks: time in min. Between *a* and *b*: 4 min.

Fig. 3. EFFECT OF CURARE ON THE NICITATING MEMBRANE. *Above: n.m.* with a 4-day-old denervation. *Below: n.m.* control. Mark on upper line: curare. Marks on middle line: acetylcholine, 1.25 μ g. in denervated side and 5 μ g. in control. Marks on lower line: time in min. Between *a* and *b*: 4 min.; between *b* and *c*: 3 min.; between *c* and *d*: 5 min.

Fig. 4. EFFECT OF ERGOTAMINE ON THE NICITATING MEMBRANE. *Above: n.m.* with an 8-day-old denervation. *Below: n.m.* control. Marks on upper line: 1 μ g. and 50 μ g. of ergotamine respectively in each side. Marks on middle line: adrenaline, 0.05 μ g. in denervated side and 0.25 in control. Marks on lower line: time in min. Between *a* and *b*: 10 min.

Fig. 5. EFFECT OF ATROPINE ON THE SUPERIOR CERVICAL GANGLION. *Above: n.m.* control. *Below: n.m.* with a 13-day-old section of preganglionic fibers. Marks on middle line: acetylcholine, 60 μ g. in control side and 40 μ g. in denervated side. Marks on lower line: time in min. Between *a* and *b*: 3 min.; between *b* and *c*: 15 min. Two mg. of atropine were injected in each side immediately after *a*.

was similarly injected in a dose of 1 μ g. As can be seen from figure 1, the blocking by atropine of the acetylcholine response disappeared first in the chronically denervated *n.m.* as compared to the normal. An earlier recovery was also observed in the denervated side. Identical results were obtained after a second injection of atropine.

PROSTIGMINE. The *n.m.* was also stimulated by acetylcholine in these experiments. Prostigmine was injected in the carotid artery of each side at a dose of 1 to 2 μg . The contraction produced by prostigmine was greater on the chronically denervated side, in agreement with the results of Rosenbluth (5). On the contrary, the potentiation to acetylcholine was lower on the denervated side (fig. 2).

CURARIZING DRUGS. The *n.m.* was stimulated with acetylcholine. The drugs used were intocostin, raw curare and β -erythroidine, injected in both carotids at the same dose and contained in equal volumes. Intocostin produced contraction in some experiments, the contraction being more pronounced on the denervated side.

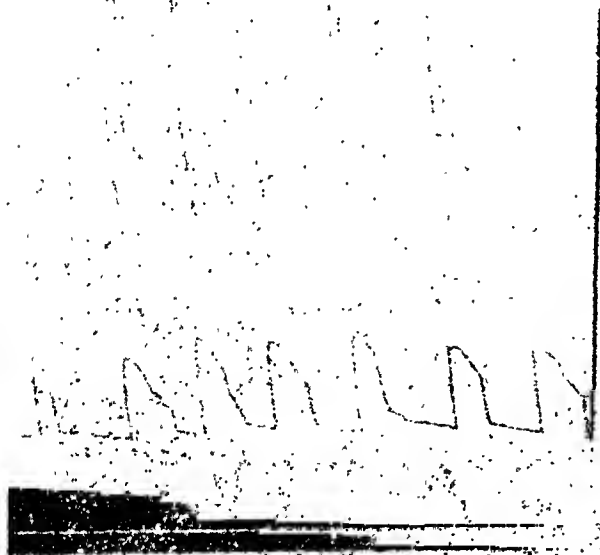


Fig. 6 (left). EFFECT OF PROSTIGMINE ON THE SUPERIOR CERVICAL GANGLION. Above: *n.m.* control. Below: *n.m.* with a 7-day-old section of preganglionic fibers. Marks on upper line: 1 μg . of prostigmine in both sides. Marks on middle line: acetylcholine, 80 μg . in control side and 40 μg . in denervated side. Marks on lower line: time in min.

Fig. 7 (right). EFFECT OF TETRAETHYLAMMONIUM BROMIDE ON THE SUPERIOR CERVICAL GANGLION. Above: *n.m.* normal. Below: *n.m.* with 6-day-old section of preganglionic fibers. Mark on upper line: 100 μg . of tetraethylammonium bromide in both sides. Marks on middle line: acetylcholine, 80 μg . in control side and 20 μg . in denervated side. Marks on lower line: time in min.

Raw curare produced in some cases a decrease in the initial tension. This decrease was of a greater intensity on the chronically denervated side. The depression of the response to acetylcholine, caused by the curarizing substances, was similar to the results obtained for atropine, i.e. of a lower intensity for the denervated membrane (fig. 3).

ERGOTAMINE AND DEHYDROERGOTAMINE. Adrenaline was used to stimulate the effectors in these experiments. The effect of ergotamine and dehydroergotamine on the response to adrenaline could not be studied because of the prolonged contraction produced by these drugs, which was of a similar character to that described for

ergotoxine by Rosenblueth (5). The contraction was always more intense in the normal *n.m.* (fig. 4). It is of interest to point out that adrenaline produced a lasting increase on the increased tension brought about by ergotamine or dehydroergotamine, i.e., it acted as a potentiator of the effect on contraction of the above-mentioned drugs. This effect was also greater in the normal side (fig. 4).

B. Sympathetic Ganglion. ATROPINE. Our experiments have shown that the blocking produced by atropine is of a lower intensity and shorter duration on the denervated *s.g.* as compared to the normal, a similar behavior to that shown by the *n.m.* (fig. 5).

PROSTIGMINE. The potentiation of the response to acetylcholine produced by this drug was greater on the normal side (fig. 6).

CURARIZING DRUGS. The administration of curare, β -erythroidine and tetraethylammonium bromide produced a blocking of the response of the *s.g.* to acetylcholine. This blocking was of a lower intensity and shorter duration in the denervated side than in the normal (fig. 7).

DISCUSSION

Our results may be considered from two different points of view: *a)* their relation to the law of denervation and *b)* the possible quantitative relations of depressing and potentiating drugs to acetylcholine.

It is clear that the law of denervation does not apply to all the drugs that have an effect on the *n.m.* and the *s.g.* The depressing effect of atropine and curare and the potentiation produced by prostigmine are of a greater intensity and longer duration on the normal side than on the chronically denervated side. Furthermore, Rosenblueth (5) observed that the sensitization to cocaine is greater in the normal *n.m.* than in the denervated *n.m.* These results differ from those expected according to the law of denervation. It would be necessary to change Cannon's wording of the law to the effect that denervation modifies the excitability of effectors, either increasing or decreasing it, to certain chemical agents. It is interesting to observe that the drugs that produce a response of the *n.m.* or the *s.g.* similar to the normal (adrenaline, acetylcholine, prostigmine in their effect on contraction) have in general an effect of greater intensity on the chronically denervated effector. On the contrary, the drugs that modify the excitability to stimulating agents show an effect of lower intensity on the denervated effector.

Concerning the drugs that increase irritability (prostigmine, cocaine), one could argue that chronic denervation exaggerates irritability to such an extent that no further increase is produced by addition of a new potentiating factor. A greater effect, therefore, would be observed on the normal side. This hypothesis cannot be applied to the case of drugs that decrease excitability. It seems more sound to give a common explanation of the phenomenon, in relation to the chemical agents that are less effective on the denervated effector.

The greater potentiating effect of prostigmine on the response to acetylcholine of the normal *s.g.* as compared to the denervated *s.g.*, may be considered in the light of the observations made by Croxatto, Huidobro and Luco (6) and Couteaux and Nachmansohn (7). According to them, denervation determines a decrease in the

cholinesterase content of the superior cervical ganglion. But, as stated above, the phenomenon seems to apply generally to the drugs that modify the excitability of the ganglion, and not only to prostigmine.

The idea that denervation produces a change in cell permeability facilitating the penetration of drugs into the cell cannot be accepted if we consider the results presented in this paper. Furthermore, with the data available at present concerning denervation any hypothesis has a purely speculative value.

Considering the second point in the discussion, it has been shown that there is no quantitative relation between the dose of acetylcholine necessary to stimulate an effector and the amount of atropine required to inhibit its response to that drug. Thus, a small amount of atropine can block the relatively high dose of acetylcholine necessary to stimulate the normal effector and cannot inhibit the response of the denervated effector to a dose 10 times lower of acetylcholine. One arrives at similar conclusions for the curarizing drugs and for the potentiating effect of prostigmine. The above considerations lead us to believe that the blocking or facilitation of the response to acetylcholine by certain drugs takes place through the participation of the biochemical systems of the cell and that these systems are changed by denervation.

Clark and Raventos (1) formulated the hypothesis that the antagonistic action of atropine on the effects of acetylcholine is due to a combination of atropine to the receptor, thus blocking the combination of acetylcholine to the same receptor. Our results do not support this hypothesis as, in the case of the denervated effector, the possibility of combination between the receptor and acetylcholine appears to be increased, whereas that of atropine to the same receptor seems to be decreased. The opposite would be true on the normal side.

SUMMARY

The effect of denervation on the excitability of the nictitating membrane and of the superior cervical ganglion, by drugs that stimulate or block those effectors, was studied in cats anesthetized with pentobarbital. The depressing effect of atropine and of curarizing drugs, and the potentiating effect of prostigmine, are of a higher intensity on the normal effector than on the denervated effector. The contraction produced by ergotamine or dehydroergotamine on the nictitating membrane is of greater intensity in the normal effector.

Results are analyzed from two different points of view: a) their relation to the law of denervation and b) the problem of the quantitative relations of acetylcholine to atropine and other depressor drugs.

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TEMPERATURE CHANGES, CONDUCTION AND ELECTRICAL SYSTOLE (Q-T INTERVAL) OF THE ISOLATED RABBIT HEART¹

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WE HAVE previously studied the influence of strophanthin and quinidine upon the perfused isolated rabbit heart under constant conditions of ionic equilibrium, perfusion time, oxygenation and temperature, 37°C. (1-3). The need for constancy in these factors has been well recognized since the classic experiments of Ringer. The effects of high and low temperatures upon the mammalian heart have been studied by numerous observers (4-6, 8, 10-12), who have noted, in general, the maximum and minimum limits of its variability at about 45° to 50°C. and 23° to 15°C., respectively. That the heart rate varies directly with the body temperature in cold- and warm-blooded animals, as well as in animal experiments (6), is also well known. The effects upon atrioventricular and intraventricular conduction times and upon the electrical systole of the isolated rabbit heart of perfusing fluids of varying temperatures, and the degree of reversibility of those effects, is the subject of this paper.

METHODS

The isolated rabbit heart was perfused through the aorta (and, hence, the coronaries) with the perfusion fluid simulating Tyrode's solution used by Calder, for the entire heart, and with the Krebs-Henseleit solution, for the isolated ventricles. Five per cent CO₂ was continuously bubbled through the reservoir; thyrotron stimulation of the auricles or of the isolated ventricles at various rates was accomplished as before (1-3). A heated water jacket kept the temperature at the desired level and this was further accomplished by actual exchange of precooled or preheated perfusion fluids throughout the perfusion system. Various orders of rotation of fluids at approximately 45°, 42°, 37°, 32°, 27°, 23° and 19°C. were used in different experiments to test reversibility and other effects. Electrocardiographic tracings corresponding to lead I were obtained, and the various EKG segments then measured with the Cambridge Measuring Instrument.

RESULTS

Effect of Cold. Prolongation of the A-V conduction time and of the Q-T interval (as well as the Q-T_e, or Q-T corrected for the rate on the basis of Bazett's constant) were noted occasionally at 34° to 35°C., fairly constantly at 32° to 33°C., at all rates of stimulation. These trends, as the tendency to spontaneous bradycardia, were

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greatly exaggerated at 27° to 28°C. Prolonged exposure at that temperature resulted occasionally in complete A-V dissociation. QRS prolongation occurred less readily. Upon ventricular stimulation at various rates, slight delay in I-V conduction was usually evident at 32°C. Upon atrial stimulation, on the other hand, it was often not evident until temperatures of 28°C. and below were reached. At 23° to 24°C. A-V dissociation, idioventricular rhythm with wide aberrant QRS waves and,

TABLE 1. EFFECTS IN REPRESENTATIVE EXPERIMENTS OF VARYING TEMPERATURES OF PERFUSION FLUIDS UPON THE INTERVALS OF THE ELECTROCARDIOGRAM

	TEMPERATURE	P-R SEC. INTERVAL	QRS SEC.	Q-T SEC.	Q-T _c SEC.	BLOCK AT R-R
<i>A. Ventricular rate of 100/min. (R-R 0.60 sec.)</i>						
1.	37°		0.052	0.22	0.32	0.15
2.	42°		0.052	0.27	0.35	0.18
3.	32°		0.058	0.30	0.38	0.18
4.	27°		0.070	0.33	0.43	0.23
1.	45°		0.036	0.23	0.30	0.12
2.	37°		0.044	0.26	0.34	0.14
3.	28°		0.059	0.031	0.39	0.17
4.	19°		0.097	0.43	0.54	0.32
1.	37°		0.046	0.29	0.38	0.18
2.	31°		0.050	0.33	0.43	0.18
3.	20°		0.070	0.41	0.53	0.18
1.	37°		0.060	0.30	0.37	0.15
2.	31°		0.060	0.35	0.43	0.20
3.	29°		0.096	0.41	0.53	0.25
<i>B. Auricular and ventricular rate of 150/min. (R-R 0.40 sec.)</i>						
1.	37°	0.122	0.035	0.18	0.31	0.18
2.	42°	0.15	0.035	0.12	0.28	0.20
3.	30°	0.235	0.035	0.19	0.32	0.25
1.	37°	0.122	0.02	0.14	0.27	0.18
2.	32°	0.210	0.032	0.19	0.34	0.28
3.	42°	0.177	0.038	0.19	0.27	0.27
4.	28°	(0.26)	(0.048)	(0.25)	0.36	0.45

finally, standstill of the heart, were the rule. Occasional hearts tolerated temperatures down to 19°C. before standstill occurred. Reversibility of these effects was usually complete as a result of changing from 33° to 37°C. (or higher), incomplete from 28° to 32°C. to 37°C., and almost absent upon replacement of solutions at 23° to 27°C. by those at 37°C. (table 1 and fig. 1).

Of some interest was the usual occurrence of paroxysmal ventricular ectopics, and ventricular tachycardia and fibrillation, at temperatures below 27°C. and at rapid rates of stimulation of the isolated ventricles. These arrhythmias appeared only

rarely when the atria were so stimulated at low temperatures, since A-V block intervened before very rapid ventricular rates of beating resulted. Markedly slowed intraventricular conduction plus extremely rapid rates of stimulation form an accepted basis for the appearance of these arrhythmias (10). No atrial arrhythmias occurred.

T-wave lowering and inversion (or reversions toward positive from negative) not infrequently followed the exposure of the heart to moderate cold. More commonly,

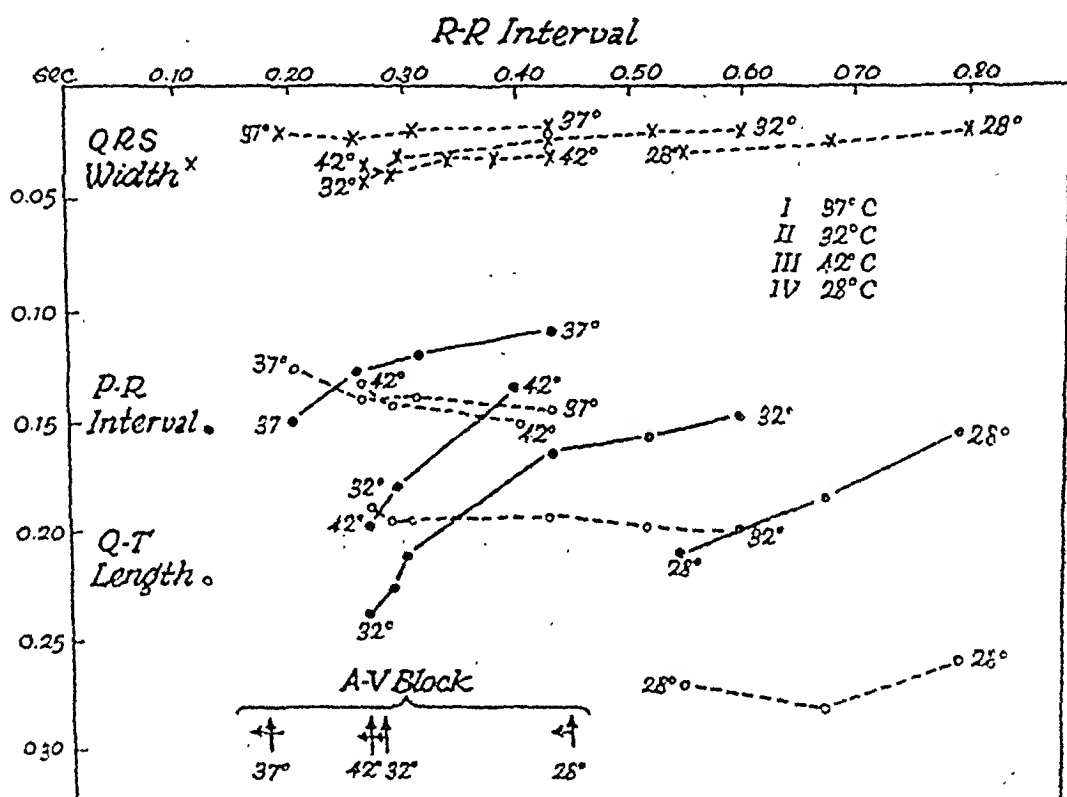


FIG. 1. THYROTROXIN-STIMULATED HEART (auricle) at rates of 75 to 300/min. Perfusion with solutions at 37°, 32°, 42°, and 28°C. in that order. Note prolongation of the intervals parallel to the degree of cooling, reversible effects on the P-R and Q-T lengths at all rates of stimulation upon heating a cooled heart.

N. B. The 'recovery curves' of A-V and I-V conductivity and of electric systole (Q-T) so obtained by artificial stimulation, including unphysiologically extreme rates, are not to be confused with the known shortening of the intervals with increasing heart rates, and vice versa, in human and animal subjects.

extreme cold caused high take off and increased height and width of the T-waves, as it caused marked widening and aberration of the QRS complex. Usually S-T prolongation occurred simultaneously with widening of the T-wave; sometimes the latter only occurred.

Effects of Heat. Perfusion at temperatures higher than 37°C. up to 40°C. and occasionally 42°C., usually shortened the P-R, QRS and Q-T intervals, especially at faster rates of stimulation. Commonly, however, perfusion of the heart at 42°C. to 45°C. resulted in progressive slowing of the atrio-ventricular conduction and prolongation of the Q-T (and of the Q-T_c) intervals. These effects quickly became irre-

versible at 45°C. on reperfusion at 37°C.; they were at least partially reversible at 40° to 42°C. QRS prolongation was occasionally absent at temperatures as high as 45°C.; sometimes delays in intraventricular conduction were evident at 42°C. (fig. 1), and more so at 45°C.; often, though, no change in, or actual increased speed of, ventricular impulse transmission occurred at that temperature (table 1 and fig. 1). Ventricular arrhythmias occurred rarely, though more frequently than at 37°C., at high temperatures, and at rapid rates of stimulation. No atrial arrhythmias were observed.

Moderate heat was frequently associated with narrow pointed T-waves, as would be expected from the more rapid rates of spontaneous ventricular beating. T-wave lowering or inversion (or reversions toward positive from negative) also occurred not infrequently from moderate heat, as from moderate cold.

COMMENT

Effects on Atrioventricular Conduction. Progressive cooling of the isolated rabbit heart resulted in consistently increasing delays in, and finally the complete failure of, conduction from the atria to the ventricles (fig. 1). Slight increases in temperature above 42°C. speeded A-V conduction, while great increases tended to slow it. These effects had been noted in intact animals (6, 10-12).

Effects on Intraventricular Conduction. Changes in temperature affected the intraventricular conduction time much less markedly than that between the atria and ventricles. This failure to influence greatly QRS width was especially evident following stimulation of the atria rather than the isolated ventricles: under those conditions marked lowering of temperature was required before intraventricular block intervened. Prolongation of the QRS even at 45°C. also occurred only infrequently. Intraventricular block has been reported on exposure of intact animals to extreme heat (10), and explained at temperatures of 60° to 70°C. on the basis of release of K^+ from the red blood cells (11). Temperatures higher than 45°C. were quickly fatal to the isolated rabbit hearts and, hence, were not employed by us.

Effects on the Q-T Interval. Lengthening of the Q-T interval and of the $Q-T_c$, parallel to the degree of cooling, was noted in all experiments. At very low temperatures the widening of the QRS and the high T-wave contributed to its prolongation, which was in all instances out of proportion to the slowing of the spontaneous rate. This effect is to be noted in the electrocardiographic tracings though not expressly stated, in several previous contributions (6, 10, 12). Moderate or marked elevation of temperature likewise prolonged the Q-T interval at various rates of artificial stimulation in our experiments; slight hyperthermia shortened it. Hyperpyrexia, in general, has been shown to prolong the $Q-T_c$ 'constant' more often than shorten it (5).

Some of the effects upon the S-T and T segments of the electrocardiogram had been noted previously (5, 8, 10) and indicate the marked lability of that recovery period of ventricular activation (Nachschwankung) under changing conditions of temperature as of other factors.

Arrhythmias. The occurrence of ventricular arrhythmias at fast rates of ventricular beating at low temperatures is easier reconciled with slowed conduction than

with the observed prolongation of the Q-T interval, since, experimentally, ventricular tachycardia and fibrillation is usually associated with a shortened refractory period (grossly represented by the Q-T length) as well as slowed conduction (9). In this connection ventricular tachycardia arising from rapid stimulation of the isolated ventricles under the influence of various drugs and ions can be most rapidly stopped by immersion of the ventricles in ice water (13). That paradoxical effect may be explained on the basis of the demonstrated marked prolongation of the Q-T interval (or ventricular refractory period). Atrial fibrillation was a rather consistent phenomenon in human subjects whose temperature was reduced to 30°C. (86°F.) (7). That it did not occur in the isolated rabbit hearts at that or lower temperatures may be explained on the basis of their freedom from nervous (vagal) or anoxic (respiratory failure) influences. Ventricular arrhythmias were encountered under conditions of hyperthermia plus rapid ventricular rates of beating in very few instances. Other workers have reported them, as well as atrial and other arrhythmias, in animals subjected to much higher temperatures (10, 11).

SUMMARY

In isolated rabbit hearts perfused with isotonic fluids at various temperatures it was demonstrated that cooling caused progressive atrioventricular and, to a lesser extent, intraventricular block, with prolongation of the electrical systole (Q-T interval) beyond the expected bradycardia influence. Excessive cold and rapid rates of stimulation of the isolated ventricles produced paroxysmal ventricular tachycardia and fibrillation, whereas, paradoxically, these arrhythmias when produced by other influences are quickly abolished by immersion of the hearts in ice water.

Heating the hearts increased the heart rate and shortened the various EKG intervals. Excessive heat, however, resulted in atrioventricular block, occasionally intraventricular conduction delay and, frequently, prolongation of the Q-T interval.

S-T and T changes occurred frequently upon cooling or heating the heart and showed the lability seen in other disturbances of homeostasis.

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HOMEOSTASIS OF POTASSIUM IN THE EXTRACELLULAR FLUID OF THE DOG DURING REMOVAL BY VIVODIALYSIS¹

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THE concentration of potassium in the extracellular fluid of the dog is normally maintained within rather narrow limits. The kidney is considered to play the dominant rôle in the maintenance of this concentration by excreting excesses and by conserving potassium during scarcity. If, however, sufficient potassium were removed from the extracellular fluid during a given period so that the quantity made available by absorption and catabolism were exceeded by an amount approximating or exceeding that in the extracellular fluid, it would be manifestly impossible for renal excretion of itself to maintain the extracellular potassium concentration substantially above zero. The presence of an appreciable concentration of potassium in the extracellular fluid under these circumstances would therefore indicate the presence of other mechanisms for the maintaining of the extracellular potassium level.

Kolff *et al.* (1) have described a dialyzer that would appear to have sufficient capacity with a reasonable working volume for blood to make feasible the removal of potassium by vivodialysis at such a rate. Their dialyzer also has the added advantage that the hydrostatic pressure difference across the membrane approximates zero at all points.

PROCEDURE

A modified form of the Kolff dialyzer was developed (fig. 1) and a suitable dialyzing fluid formulated (table 1). Male dogs of varied ancestry and condition were used. They were kept anesthetized with sodium pentobarbital while being dialyzed (25 mg/kg. intraperitoneally initially and then 6 mg/kg. intravenously as needed). Heparin was given intravenously to prevent clotting (10 mg. plus 2 mg/kg. initially and then 0.2 mg/kg. every half hour).

The blood samples of 30 to 50 cc. generally were drawn into the syringes shown on the dialyzer. After a portion had been saved out for hemoglobin determination, the remainder of the sample was centrifuged under light mineral oil to separate the plasma. This was stored anaerobically in a syringe kept in a refrigerator until the carbon dioxide content had been determined. The sample was then transferred to a test tube and handled aerobically. If only potassium and hemoglobin were to

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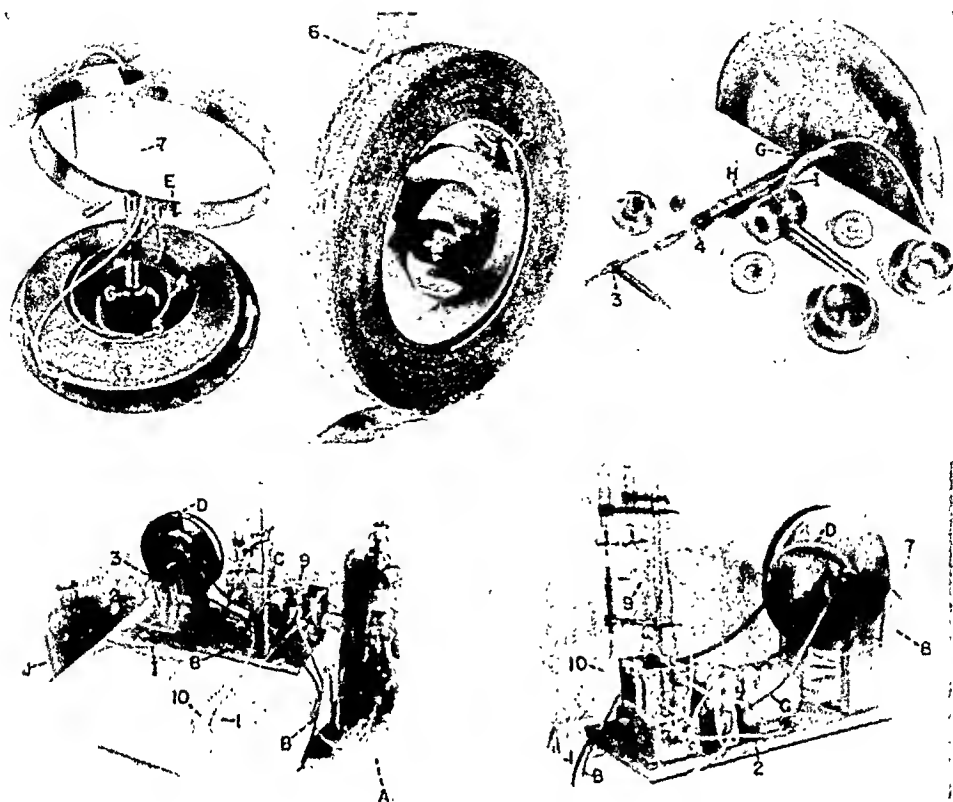


Fig. 1. THE DIALYZER consisted of a verticle Archimedian spiral of cellulose tube revolving partly submerged in a dialyzing bath. The blood flowed through the tube by gravity in waves near the bottoms of the turns as the spiral rotated. The strip of 25 mesh/in. screen supporting the spiral had wire helices fastened on its edges to separate the turns about an $\frac{1}{4}$ in. A tube-pump (Sigma-motor Mfg Co., Niagara Falls) transported the blood both to the spiral and back to the subject. It also brought fresh fluid to the bath. An Archimedian pump in the core of the spiral kept the surface of the bath at the lower margin of the core. A $\frac{1}{4}$ hp. motor drove the pump and turned the dialyzer at 18.6 R.P.M. The metal parts of the dialyzer were of monel or stainless steel. All nuts were threaded so they would tend to tighten in operation. Sponge rubber gaskets made the case water-tight and held the spiral in place. The path of the blood is shown by the arabic numerals and that of the dialyzing fluid by the letters taken in order. Fifty ft. of cellulose tube (Visking Corp., Chicago) was used to wind the spiral. It had an inflated diameter of $\frac{3}{8}$ in. A new tube was used for each dialysis. It was kept wet and stretched during winding. Connectors of glass tubing were tied into the ends with several turns of tightly stretched rubber band. These were anchored to the supporting screen with stretched rubber bands, which would take up any slack developing later. Polyethylene tubes of 0.1 in. bore and 0.0148 in. wall were used to carry blood in the rest of the system. They were joined both to each other and to the glass and metal parts by connectors of polyvinyl chloride plastic tube of $\frac{1}{8}$ in. bore. These were held on by everted collars formed on the ends of the polyethylene tube by heating them in an open flame for a moment. The connectors, the glass and metal parts, and the amber gum tube in the pump were treated with Dri-Film (2) if they were to come in contact with blood. A small quantity of D.C. Antifoam (Cov Corning, Midland, Mich.) was spread inside of the hubble and clot trap in the line returning blood to the animal. The pump was arranged to have a greater capacity for removing blood from the dialyzer than from the subject. One liter/min. of 5% CO_2 in O_2 was passed into the dialyzing fluid line between the pump and the dialyzer. The assembled system was cleaned by filling it with 1/500 Zephiran solution, which was later thoroughly washed out with sterile saline. The operating volume was 300-400 cc. for blood and about 1 L. for dialyzing fluid. Lengths of Polyethylene tube of 0.082 in. O.D. and 0.007 in. wall were inserted into either the femoral or external jugular vein through no. 12 hypodermic needles. After the tubes were started the needles were stripped off. The tubes were then advanced into the vena cava. Their protruding ends were connected to the lines from the machine by polythene adapters formed from the 0.1 in. I.D. tubing by heat and pressure. Care was taken to place the tubes in the vena cava so as not to withdraw blood just returning from the dialyzer.

be determined, only 10 cc. of blood was taken and this was handled aerobically. Hemoglobin was estimated as described previously (5). Carbon dioxide was estimated manometrically (6). Chlorides were determined by the titration of Schales and Schales (7) on the filtrate obtained by adding 1 part of plasma to 10 parts of tungstic acid made by adding 1 volume of 10 per cent sodium tungstate and 1 volume of $2/3$ N sulfuric acid to 8 volumes of water. Inorganic phosphorus was estimated by a modification of the method of Fiske and Subbarow (8). Three cc. of filtrate obtained by adding 1 part of plasma to 10 parts of 10 per cent trichloroacetic acid

TABLE I

FORMULA FOR DIALYZING FLUID		COMPOSITION	
	gm.		
Sodium chloride	134.0	Na	333 mg. %
Potassium chloride	5.7	K	15.0 "
Calcium chloride dihydrate	4.7	Ca	6.53 "
Magnesium lactate trihydrate	3.4	Mg	1.61 "
Citric acid	0.6	Cl	432 "
Glucose	20.0	CO ₂	66.6 vol. %
Carbowax 6000 W	200.0	P	4.61 mg. %
Sodium dihydrogen phosphate mono-hydrate	4.1	Lactic acid	11.9 "
Sodium bicarbonate	50.0	Citric acid	3.00 "
Water—sufficient to make 20 liters		Glucose	100 "

In the potassium removal experiments 4.5 gm. of sodium chloride was substituted for the potassium chloride. To prepare the solution, the dry components other than the bicarbonate for any convenient number of batches were thoroughly mixed together. Quantities of the mix for 20-liter batches were weighed out and stored in airtight containers. A 20-liter batch of the dialyzing solution was then prepared by dissolving one of these in about 18 liters of water, adding the bicarbonate, which had been dissolved in another liter of water, and diluting to volume. Adequate stirring with an avoidance of turbulence was necessary to avoid loss of carbon dioxide gas. The finished solution was warmed to 40–45° C. just before it was to be used. This sufficed to keep the temperature in the dialyzer at about body temperature. The formula was calculated on the basis of the data of Greene and Power (3). Lactate and citrate in physiological amounts were used to supply part of the undetermined anion and bicarbonate the rest. *Carbowax 6000 W* is the trade name under which the Carbide and Carbon Chemical Company supplies a polyethylene glycol with the average molecular weight of about 6,000. This substance penetrated the dialyzing membrane used only very slowly and served to balance the colloidal osmotic pressure of the plasma proteins (4).

was measured into a test tube. A concurrent blank and standard were prepared by measuring 3 cc. of a zero and a 5 mg. per cent standard into second and third test tubes respectively. The zero standard was prepared by adding 1 part of water to 10 parts of 10 per cent trichloroacetic acid, and the 5 mg. per cent standard by adding 1 part of a solution of potassium dihydrogen phosphate containing 5 mg. per cent of phosphorus to 10 parts of 10 per cent trichloroacetic acid. Three cc. of color reagent was then added to each of the tubes. The contents were thoroughly mixed and after standing about an hour were compared in a photoelectric colorimeter at the 660 mμ wave length. The color reagent was freshly prepared by adding 4 parts of the aminonaphthol sulfonic acid reagent and 10 parts of molybdate II to 36 parts of water. The premixing of these reagents was a convenience and seemed to result

in blanks with less color. A couple dozen analyses can be made concurrently with this procedure with good checks. Calcium was determined by the Clark Collip procedure (9). Two cc. of 2 per cent ammonium oxalate was added to 2 cc. of plasma. The precipitate was washed twice with 2-cc. portions of ammonia water instead of once with 3 cc. Magnesium was estimated in a 3-cc. portion of the calcium-free supernatant fluid by the method of Denis (10). The washed ammonium magnesium phosphate precipitate was dissolved in 3 cc. of the zero standard from the phosphate method and compared with a 10 mg. per cent standard prepared analogously to the 5 mg. per cent standard described. The checks were not close and the results must therefore be regarded as approximate.

TABLE 2

PTS. OF K ADDED/ 60 PTS. LITHIUM	60 MG. LITHIUM/L.	60 MG. LITHIUM/1.1 L.	60 MG. LITHIUM/L. UNUSED DIALYZING FLUID PLUS 0.1 H ₂ O	10 PTS. USED DIALYZING FLUID PLUS 1 PART SOLU- TION OF 600 MG. LITHIUM/L.	1 PT. PLASMA PLUS 10 PTS. A SOLU- TION OF 60 MG. LITHIUM/L.	1 PT. DILUTED URINE PLUS 10 PTS. A SOLUTION OF 60 MG. LITHIUM/L.	PTS. SODIUM ADDED/ 60 PTS. LITHIUM	60 MG. LITHI- UM/L.	1 PT. OF PLASMA PLUS 100 PTS. A SOLUTION OF 60 MG. LITHIUM/L.
	Photometer reading			Potassium found mg./l. Total Added					
								Photo- meter reading	Sodium found mg/l. Total Added
50	100	100	96.2				50	100	
40	80	79.4	79.6		41.4 38.4	44.6 38.4	40	80.8	
30	59	58.4	58.9	46.2 26.6	31.9 28.9	34.0 27.8	30	59.6	
20	39.5	39.3	40.6	38.0 18.4	22.5 19.5	24.9 18.7	20	40.4	
10	19.2	19.5	22.4	28.3 8.7	12.2 9.2	14.6 8.4	10	20.4	46.7 10.4 (46.6) (10.1)
5	9.4	9.3	10.6	24.4 4.8	7.96 4.98	9.8 3.6	5		
0			0	19.6	2.98	6.2	0		36.3 (36.5)

Sodium and potassium were estimated with the model 52A Perkin and Elmer flame photometer, which employs the internal standard principle (11). The plasma sodium was determined by addition of 0.1 cc. of plasma to 10 cc. of a lithium chloride solution containing 60 mg. of lithium per liter and comparing the resulting solution with a series of sodium and lithium chloride solutions containing 60 mg. of lithium and 10 to 50 mg. of sodium per liter. Potassium was determined in the same way except that 1 cc. of plasma was used instead of 0.1 and the standard series was extended to include the 5 mg. liter concentration. The used dialyzing fluid from the potassium removal experiments was analyzed for potassium by adding 10 cc. to 1 cc. of a lithium chloride solution containing 600 mg./liter of lithium and comparing the resulting solution with a series of solutions made by adding 10 cc. of unused dialyzing fluid to 1 cc. of a solution of potassium and lithium chlorides containing 50 to 500 mg. of potassium and 600 mg. of lithium per liter. Two to five weeks after the potassium removal the animals were again allowed to fast for two days and then anesthetized as before. Their bladders were washed with distilled water. They

TABLE 3

EXPER.	TIME, MIN.	HEMOGLOBIN, GM. %		Na, MG. %		K, MG. %		Ca, MG. %		Mg, MG. %		Cl, MG. %		CO ₂ , VOL. %		INORGANIC PHOSPHORUS MG. %		TOTAL CATION, mEq.		TOTAL ANION, mEq.		UN- DETER- MINED ANION mEq.	
		dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.	dog	dial.
1	-10	11.8	338	15.9	10.6	1.4	363	58.3	4.4	158	130	28											
	43	11.4	335	13.9	10.2	1.5	2.2	365	55.4	4.8	156	129	130	27	29								
	246	11.6	335	14.8	10.1	1.4	1.5	368	53.6	5.8	156	131	134	25	25								
	272	11.8	342	15.1	9.9	1.4	379	54.9	6.0	159	134	25											
2	2	16.1	389	14.0	11.6	1.5	400	55.1	3.6	180	138	42											
	127	17.9	375	13.7	11.4	1.6	0.9	389	51.9	4.1	174	175	134	40	41								
	284	17.7	371	16.2	10.3	1.8	1.8	389	57.0	4.0	172	179	136	36	41								
3	-100	10.1	364	18.5	10.7	1.6	391	54.1	4.4	169	138	31											
	10	9.6	9.6	14.1	5.0																		
	40	10.4	10.4	16.0	5.0																		
	70	10.4	10.6	15.2	5.1																		
	110	11.9	352	14.8	10.4	1.1	1.3	402	55.8	6.2	162	167	141	21	27								
	155	11.3	368	15.0	4.8																		
	188	10.8	11.0	15.2	4.5																		
4	230	10.1	10.4	14.1	5.4																		
	4	11.2	385	13.0	9.6	1.7	411	48.6	4.1	177	139	38											
	30	11.2	11.2	14.6	3.2																		
	105	10.7	10.7	12.0	2.8																		
	160	10.8	10.7	11.6	3.0																		
	240	10.8	10.8	12.2	4.0																		
	308	11.3	373	12.0	9.9	10.0	1.7	2.0	400	382	58.2	54.6	5.8	4.9	172	170	141	134	31	36			

TABLE 3—Concluded

5 K removal 275 min. of dialysis	1	15.4	36.4	15.2	9.7	1.3	399	52.0	4.1	168	137	31										
	32	15.6	15.4	14.7	3.1																	
	88	14.8	14.8	13.5	4.0																	
	162	15.4	15.4	12.7	3.7																	
	273	16.0	15.8	36.4	36.3	11.4	3.2	9.8	9.7	2.4	2.2	398	400	53.2	55.5	6.9	5.5	168	165	139	140	29
8 K removal 290 min. of dialysis	-9	13.2	36.2	16.2	10.4	1.2	405	55.8	3.7	168	140	28										
	21	13.7	13.9	16.0	5.0																	
	119	15.6	15.8	12.5	4.0																	
	190	16.0	16.0	11.4	2.8																	
	285	15.0	15.0	36.6	36.7	11.5	2.5	10.3	10.2	2.1	3.2	394	395	55.6	55.9	6.7	5.5	169	168	138	138	31

Other than for hemoglobin the values presented are for plasma. Time was counted only while blood was being pumped from the dog. The first sample in any case was taken before any blood had returned from the dialyzer to the dog. Columns headed *dog* and *did.* show values for samples coming from the dog or dialyzer respectively. For the purpose of calculating total anion in milliequivalents the molarity of carbon dioxide was multiplied by 0.951 and that of inorganic phosphorus by 1.8.

were allowed to continue under the anesthesia for a period equal to the elapsed time of the dialysis in each instance. Their bladders were then washed a second time with distilled water and the washings diluted to one liter. One cc. aliquots of the diluted washings were analyzed for potassium by the method used for plasma. These methods for determining sodium and potassium were checked by determining known added amounts of these elements. The results of these checks are shown in table 2. It appears that the methods for potassium yield values that are probably somewhat lower than the true values but which are nevertheless useful for the purposes of this study.

RESULTS AND DISCUSSION

The results shown in table 3 indicate that the plasma components determined other than potassium behaved in substantially the same way in both the control and potassium removal experiments. Inorganic phosphorus tended to rise with

TABLE 4

EXPER.	WEIGHT	PUMP RATE		ELAPSED TIME OF DIALY- SIS	VOL. OF DIALYZ- ING FLUID	PLASMA K		CALCU- LATED INITIAL EXTRA- CELLULAR K	K REMOVED	K EX- CRETED IN URINE	PRIOR FAST
		Blood	Dialyz- ing fluid			Initial	Final				
	kg.	cc./min.	cc./min.	min.	l.	mg. %	mg. %	gm.	gm.	gm.	days
1	43.2	110-119	293	270	79.5	15.9	14.8		control		0
2	21.9	102	274	288	80.0	14.0	16.2		control		2
3	41.8	93	288	243	72.9	18.5	14.1	2.32	1.26 ¹		2
4	27.2	67-93	252	321	79.5	13.0	12.0	1.06	1.23	.254 ²	2
5	17.3	99-108	271	288	79.5	15.2	11.4	0.79	1.42	.069	2
6	26.4	100-104	271	309	79.5	16.2	11.5	1.28	1.54	.070	2

The elapsed time of dialysis includes the time during which the dialysis may have been briefly interrupted. The initial extracellular potassium was calculated by multiplying the plasma concentration by 0.3 of the body weight. It seems fairly certain that the extracellular fluid of the dog does not exceed this fraction of the body weight (4, 12-14) and that its potassium concentration does not exceed that in the plasma (15).

¹ The true value is probably nearer 1.5 gm., for about $\frac{1}{2}$ of the used dialyzing fluid was lost and the potassium it presumably contained was not included.

² Probably higher than normal. The animal had contracted distemper and died the next day.

time in both but there is no evidence that it entered the plasma from the dialyzing fluid. The other ions seemed to remain fairly constant throughout the dialysis and did not exhibit any evidence of net transport across the dialyzing membrane. The hemoglobin varied somewhat, but this seems to have been related to the response of the subjects to the hemorrhage (the 300-400 cc. of blood needed to operate the dialyzer) and not to the net transport of water across the dialyzing membrane. Potassium was effectively removed by the dialyzer when it was omitted from the dialyzing fluid. The subjects, however, were able to maintain levels approaching low normal in spite of this removal. The results presented in table 4 show that the potassium found in the used dialyzing fluid approximated or exceeded the total that would be expected in the extracellular fluid at the beginning of the experiment. The amounts recovered in the urine during a comparable period of anesthesia and

after a similar period of fasting correspond to what was expected from those reported in the literature (16) and are too small to account for much of that removed by dialysis. It follows therefore that according to the postulate set up in the introduction that some mechanism or mechanisms other than renal excretion are involved in maintaining the concentration of potassium in the extracellular fluid within normal limits.

The physiological rôle of potassium has been extensively reviewed (17-19). It will suffice here to mention the finding of Winkler and Smith (13) that the volume of distribution of injected potassium is greater than that of the extracellular fluid in the dog. This indicates that factors other than renal excretion are of importance in preventing excesses of potassium from elevating the extracellular fluid concentration unduly while conversely the results of the present study show that the effects of potassium removal are also minimized by mechanisms other than renal excretion.

SUMMARY

Amounts of potassium approximating or exceeding the amount calculated to be in the extracellular fluid initially were removed from dogs within four to five hours by vivodialysis.

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RESPIRATORY WATER VAPOR AT SIMULATED ALTITUDE

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THE desirability of obtaining information concerning the respiratory water vapor loss at altitude became evident while testing oxygen breathing equipment in the decompression chamber. The apparent facilitation of freezing of exhalation valves observed at altitude could be caused by either increased vaporization in the lungs due to alterations in the physical characteristics of the respired gases, or increased respiratory ventilation, or a combination of these factors.

In making corrections for the water vapor content of respired gases, respiration physiologists assume a partial pressure of 47 mm. Hg, or that of pure water at body temperature. The accuracy of this value is obviously open to question, since evaporation into the alveolar space is not from surfaces of pure water of which the precise temperature is known. If altitude affects the amount of water vapor in the alveolar space, the magnitude of the change becomes increasingly significant the smaller the total barometric pressure. Since at present the measurement of the degree of saturation within the alveolar spaces is impractical, we felt that some indication of whether or not it is affected by altitude might be found in studies of exhaled water vapor.

The most frequently used method of determining exhaled water vapor has been to weigh the amount of water absorbed by suitable chemicals over which the breath has passed (1-5). The disadvantage of this method is the effect upon the normal respiratory pattern of the high resistance introduced into the airway, which alters velocity and concomitantly the time relationships. Dew point determinations of the exhaled air have been made by Lasage (6) and Christie and Loomis (7). With this method there is the possibility of the end point being obscured by particulate water carried out with the air when expiration occurs at a high velocity. Wet and dry thermocouple readings have been attempted in the upper respiratory passages by Seeley (8), and in the bronchi by Fugitt (9). Here the difficulty of shielding the junctions without interfering with the airflow past them and the continually changing velocity of this flow make reliable determinations infrequent. Lombard (10) secured a few estimations of lung water loss by subtracting the body weight lost while holding the breath from the total insensible weight lost over a comparable period.

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Our vapor pressure estimations are based on measurements made with *a*) a gravimetric and *b*) a dew point apparatus. The first method involved freezing out and weighing the water contained in a measured volume of exhaled air. This method was developed independently of Burch's work (11), which is based on the same principle. The second method depended upon determination of the temperature at which the water vapor in the exhaled air started to condense. This dew point determination was independent of air volume measurements and had the additional advantage of speed and directness. Both types of apparatus were designed to minimize dead space and resistance, and were used concurrently.

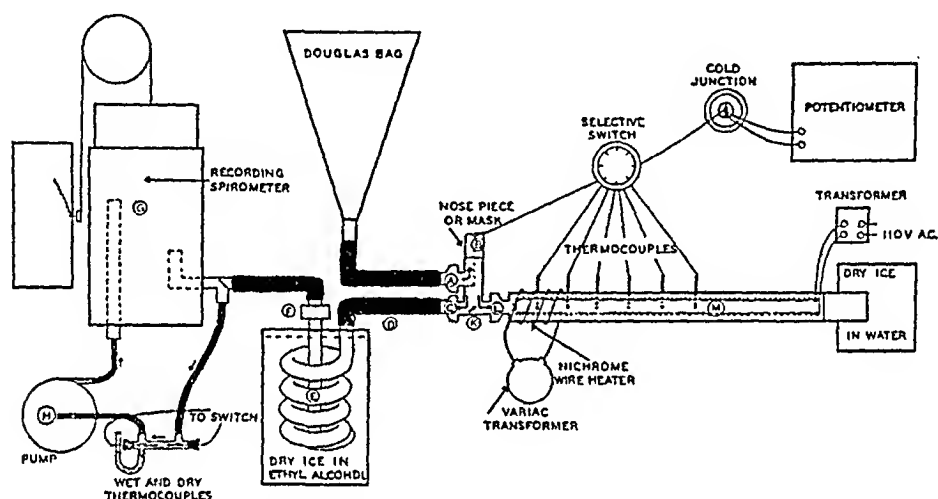


Fig. 1. SCHEMATIC DIAGRAM OF APPARATUS. (A), Plastic inspiratory valve; (B), rubber nosepiece; (C), metal expiratory valve; (D), corrugated rubber tubing; (E), chilled 'scrubber'; (F), metal housing for fine wire mesh; (G), recording spirometer; (H), airtight pump for recirculation of gas; (K), hand-operated valve to direct gas through gravimetric or dew point apparatus; (L), metal expiratory valve; (M), copper bar covered with enclosed space, glass top of which is kept from fogging by heated coil.

METHODS

Gravimetric Apparatus. Figure 1 is a schematic diagram of the apparatus. Inhalation was through a rubber check valve in plastic housing (A) mounted on a metal tube onto which was fitted the nosepiece (B) made of rubber tubing cut to fit across the upper lip and flare of the nostrils, and cushioned with sponge rubber. A metal septum separating the air streams extended to within 1.5 cm. of the nasal septum, making a dead space within the apparatus of approximately 7.5 cm.³ The exhaled air passed through a metal-bodied check valve (C) and corrugated rubber tubing (D) to a 'scrubber' (E) where the water vapor was frozen out. Premature condensation was prevented in the metal tube and exhalation valves (C or L) by heating the metallic parts so that they felt warm to the touch. Condensation in the rubber nosepiece (B) was never visible, and the small amount occurring was assumed to have reached equilibrium by the time the first measurement was made. The scrubber, a brass canister with internal baffle arrangement or alternatively a copper coil, was immersed in ethyl alcohol chilled with dry ice to approximately -50°C . In

our early experiments, escape of frost was prevented by a plug of glass wool placed near the outlet of the canister. The glass wool offered noticeable resistance to expiration, and it was later replaced by a large area of fine mesh screen (F) such as that described by Burch (11). The cold dry air then passed into a recording spirometer (G).

The accuracy of the final results was dependent upon reliable gas volume readings. By means of an airtight pump (H), the spirometer gas was recirculated past wet and dry thermocouples for 5 minutes at the end of each experiment (total spirometer volume recirculated approximately three times), when the lowest dry thermocouple temperature was reached, indicating thorough mixing, and the relative humidity was 95 per cent or greater. At this time the volume reading was made.

The mean pressures developed at the nose during eupnea were -1.2 mm. Hg for inspiration and $+0.9$ and $+2.7$ mm. Hg for expiration through the wire mesh and the glass wool respectively.

The gain in weight of the scrubber with attached parts (D, E and F, fig. 1) represented water vapor collected during the test period. Composition of the gas within the scrubbers had to be identical in the initial and final weighings, except for water vapor. This was accomplished by ventilating the scrubbers with 6 liters of dry room air after the experiment. Open drying tubes filled with indicating Drierite were attached to the scrubbers to ensure pressure equilibration during altitude and temperature changes without loss of collected water or addition of ambient water vapor, and were included in the weighings.

After an experiment, the exterior of the scrubbers was dried at room temperature under a fan, and they were kept in desiccators for at least 4 hours before weighing. Before being used again they were dried internally with warm air and kept in desiccators overnight. A beam balance sensitive to 5 mg. with 1-kg. load was used. The weights were read to the nearest 5 mg. which represented a sensitivity of 0.5 per cent with the mean net weight found in our experiments.

Calibration of the canisters and coils with known amounts of water (range 705-1740 mg.) showed a mean recovery of approximately 97 per cent, the mean difference and standard error between sought and found weights of water being $-2.69 \pm .54$ per cent. The vapor tensions of water at -50°C. and of calcium sulfate do not entirely account for the consistently low recovery. In addition, a significant loss of aqueous vapor seems to have occurred when the scrubbers were disconnected and stoppered.

All calculations are based on the Smithsonian Meteorological Tables (1931). Computation of the amount of water per unit volume of respired gas involves correcting the exhaled gas in the spirometer to standard conditions of temperature and dryness and to the corrected ambient pressure, that is, 760 or 226 mm. Hg, according to whether or not the test was made at ground level or at 30,000 feet simulated altitude. This ambient pressure correction is indicated by inserting the letter 'a' into the usual STPD symbol for standard conditions. The weight of water per liter STPaD is a physiologically artificial value, but one which expresses aqueous vapor changes in terms of constant gas volumes. The physiological condition is expressed more appropriately by further correcting the standard volumes to exhaled conditions. In our series, this is 80 per cent saturation at 32.2°C. , the mean of 52

thermocouple measurements of the highest temperature observed at the nostrils during exhalation, standard deviation $\pm 0.62^{\circ}\text{C}$. The value for saturation is that most frequently found in the literature for exhaled air. Two disadvantages of this correction are apparent. In the first place, a constant value is assigned to the vapor tension, variations of which are under investigation. In the second place, the disproportionate corrections for moist volumes at total pressures of 760 and 226 mm. Hg respectively obscure the small changes in weight of water which were found.

Dew Point Apparatus. The preceding discussion makes apparent the advantage of a method of determining aqueous vapor pressures without recourse to calculations based on gas volumes. Accordingly, direct measurements of dew points were made. The apparatus, which was designed by Mr. Howard F. Brubach of this Laboratory, consisted of a massive copper bar (M, fig. 1), $5 \times 5 \times 70$ cm., one end of which was cooled by dry ice in water, while the other end was heated with a nichrome wire, the temperature of which was controlled by a Variac transformer. Adjustment at the heated end maintained a temperature gradient from approximately 16° to 35°C . along the calibrated length of the bar. By means of a manually operated valve (K), the exhaled air was directed by way of the heated metal-bodied check valve (L) through an enclosed space running the length of the highly polished bar surface. A heated wire coil prevented condensation on the glass top so that the bar surface remained visible. Condensation of vapor began where the bar temperature was at the dew point of the gas. This temperature was measured by means of iron-constantan thermocouples inserted into holes drilled just below the bar surface at regular intervals. The thermocouples were made of no. 34 B and S gauge wire welded at the exposed tips and had tin-foil tamped around them to assure good contact with the bar. The e.m.f. was measured by means of a Leeds and Northrup type K' potentiometer with built-in galvanometer, using a cold junction maintained at 0°C .

The effects of changes in gas velocity upon surface temperature and of cooling due to evaporation were minimized by the massiveness of the bar. During inspiration, when no gas was flowing across the bar, condensation could be seen moving towards the warm end (indicating a rise in vapor pressure), apparently due to warming of the relatively stationary column of gas in contact with the bar plus evaporation from the moisture droplets at the cold end. As expiration began, this faint fogging of the bar retreated towards the cold end, until it merged with the very definite 'meniscus' of heavy condensation lower down, the distance on the bar corresponding roughly to a decrease in temperature of 2°C . If exhalation was unduly prolonged, this meniscus was seen to creep towards the warm end, due to the increase in vapor tension with increasing admixture of alveolar gas. The increase in aqueous vapor pressure during slow, deep respirations compared with that during rapid, shallow breathing could also be demonstrated.

An additional thermocouple was inserted between the nostrils and nosepiece. By closing the galvanometer circuit only toward the end of exhalation, excursions of the galvanometer were kept to a minimum. Since a slow respiratory rate would afford the galvanometer more time to approximate the maximum deflection, we tested statistically whether or not the magnitude of the measured temperature depended upon the rate of respiration. Twenty-four observations of rate and tem-

perature at ground level showed an insignificant correlation coefficient of $-.26$. It appears, therefore, that our thermocouple placed at the nostrils accurately measured the maximum temperature of exhaled gas within the limits imposed by the wire size and physiological conditions.

Two other thermocouples furnished wet and dry temperature readings of the circulating spirometer gas. The one-half inch tip of exposed wires of the wet thermocouple, placed downstream from the dry thermocouple, was kept adequately moistened by wrapping it with a strip of long-fibred lens paper the end of which dipped into distilled water. The iron wire was kept free from rust.

To test the accuracy of the dew point apparatus, dew point readings were compared with simultaneous wet and dry thermocouple measurements while gas was being passed intermittently across the bar. Agreement was within approximately one per cent when tested with room air, spirometer air, and He-O₂ gas mixtures, vapor pressures of which ranged from 14 to 24 mm. Hg.

Subjects. Twelve of the subjects were Navy volunteers 17 to 19 years of age, in good health and free of respiratory infection at the time of our experiments. They had had one indoctrination chamber 'flight' before serving as subjects. Three subjects were laboratory personnel, older, and well versed in decompression chamber technique. Since earlier work (12) has shown that variations from day to day and between individuals are as large as variations due to different conditions, the experiments were designed for paired observations.

PROCEDURE AND RESULTS

Altitude Changes. Fifteen subjects were tested at ground level and simulated altitude while breathing dry oxygen from Douglas bags. All tests were made at room temperature and humidity and with the subjects comfortably clothed and sitting quietly in the decompression chamber. After a short rest, and then breathing dry oxygen from a mask and demand regulator for 5 minutes, the subject was transferred to the nosepiece and after 2 minutes a dew point reading was taken. Then the exhaled gas was directed through the canister or coil, which had been immersed in the chilled alcohol 5 minutes previously, and into the recording spirometer. When the spirometer was filled (3-5 minutes), it was turned out of the system and recirculation of its volume started. During this time a second dew point reading was taken. Upon completion of the ground level run, the subject was transferred back to his face mask and the ascent to 30,000 feet simulated altitude was made at the rate of 2,000 feet per minute. As soon as the decompression chamber was leveled off the experiment was repeated.

Table 1 shows the respiratory data and the vapor pressures calculated from them and from the dew points. Mean differences between the ground level and altitude values are shown, and for the vapor pressures the standard errors of the differences have been calculated. The probability that the differences between the experimental and control values occurred by chance was determined by Fisher's *t* test.

The average weight of respired water lost per minute was lower at 30,000 feet simulated altitude than at ground level. This decrease occurred in 14 of the 15 subjects. The one subject (no. 18) who showed an increase in minute water output

TABLE 1. RESPIRATORY WATER VAPOR AT GROUND LEVEL AND 30,000 FEET SIMULATED ALTITUDE
15 subjects at rest breathing oxygen

SUBJECT NO.	EXPERIMENTAL CONDITION	RESP./ MIN.	WATER LOSS/ MIN.	MINUTE VOLUMES		VAPOR PRESSURES			EXHALED GAS TEMPERATURE
				STPaD ¹ Correction	Exhaled ² Correction	From STPaD Vols.	From Exhaled Vols.	From Dew Points	
			mg.	l.	l.	mm. Hg	mm. Hg	mm. Hg	°C.
13	Ground Altitude	14.7	176	6.37	7.40	28.9	24.5	29.2	
		13.0	123	4.68	6.02	27.4	21.0	25.2	
14	Ground Altitude	15.3	303	9.24	10.75	34.5	29.4	31.0	
		13.4	201	5.54	7.12	38.4	29.6	28.9	
16	Ground Altitude	13.6	191	6.41	7.45	31.3	26.6	30.7	
		15.6	138	4.43	5.70	32.8	25.1	28.7	
17	Ground Altitude	13.1	301	8.35	9.71	38.2	32.6	32.5	
		13.7	218	5.82	7.47	39.7	30.4	30.0	
18	Ground Altitude	13.3	167	7.26	8.44	23.6	20.2	25.3	
		10.6	186	5.74	7.38	34.1	26.1	25.2	
19	Ground Altitude	11.4	276	8.51	9.89	34.1	29.0	30.8	
		10.5	241	6.75	8.68	37.8	29.0	29.7	
21	Ground Altitude	5.8	195	5.48	6.38	37.8	32.0	31.8	
		7.4	153	4.13	5.31	39.3	30.1	29.6	
22	Ground Altitude	20.0	358	13.79	16.04	26.9	22.9	31.3	
		21.8	322	10.97	14.09	30.8	23.5	31.3	
27	Ground Altitude	17.0	180	6.47	7.53	29.0	24.8		28.1
		14.6	129	4.14	5.32	32.6	24.9		30.9
28	Ground Altitude	13.0	363	9.96	11.58	38.6	33.0		32.0
		10.9	285	7.72	9.93	39.3	30.1		26.9
31	Ground Altitude	14.5	240	6.54	7.61	38.8	33.2		34.0
		13.4	191	4.66	5.99	43.9	33.5		34.4
33	Ground Altitude	13.5	242	6.78	7.88	37.8	32.2		31.5
		13.8	148	4.30	5.52	36.3	27.9		30.8
303	Ground Altitude	14.1	176	7.69	8.94	23.6	20.1		
		13.6	156	6.34	8.15	25.7	19.7		
305	Ground Altitude	14.2	255	9.54	11.10	27.7	23.9		
		14.0	220	7.95	10.22	28.9	22.3		
307	Ground Altitude	11.8	209	7.16	8.33	30.6	25.8		
		14.8	175	6.31	8.11	29.0	22.3		
Average	Ground Altitude	13.7	242	7.97	9.27	32.1	27.4	30.3	31.4
		13.4	192	5.96	7.67	34.4	26.4	28.6	30.8
Mean difference & S. E.....		-0.3	-50	-2.01	-1.60	+2.3±0.8	-1.0±0.6	-1.7±0.5	-0.6
Percentage difference.....		-2	-21	-25	-17	+7	-4	-6	-2
P value ³01-.02	.1-.2	.001-.01	

¹ Volumes corrected to standard conditions: 0°C., 760 or 226 mm. Hg pressure, dry.² Volumes corrected to exhaled conditions: 32.2°C., 760 or 226 mm. Hg pressure, 80% saturated.³ P designates the probability, as determined by Fisher's *t* test, that the differences between the experimental and control values are arrived at by chance.

at altitude also markedly slowed his respirations. The average respiratory rate was not significantly altered at altitude, but this mean value does not faithfully indicate the individual responses, for all except 2 subjects either increased or decreased their respiratory rates at altitude compared to their respective ground level values.

The minute volumes consistently decreased at altitude, the magnitude of the decrease depending upon whether or not the volumes were corrected to dry or to exhaled conditions. The mean dry volume (STPaD) decreased at altitude more than did the mean water loss (25% compared to 21% decrease). This resulted in an increase at altitude in the mean vapor pressure calculated from this dry volume, the increase averaging 2.3 mm. Hg, range -1.5 to $+10.5$. Individually, 12 subjects showed this increase at altitude. On the other hand, the decrease at altitude in the mean minute volume corrected to exhaled conditions was smaller than the decrease in mean minute water loss (17% compared to 21%), so that the mean exhaled vapor pressure was therefore lower than at ground level. Five subjects out of the group

TABLE 2. RESPIRATORY WATER VAPOR BREATHING OXYGEN AND OXYGEN-IN-HELIUM MIXTURES
Mean values of 8 resting subjects at ground level.

	RESP./MIN.	WATER LOSS/MIN. mg.	MINUTE VOLUMES		VAPOR PRESSURES			EXHALED AIR TEMPERATURE °C.
			STPD ¹ correc- tion	Ex- haled ² correc- tion	From STPD vol.	From exhaled vol.	From dew points ³	
			l.	l.	mm. Hg	mm. Hg	mm. Hg	
Oxygen....	12.3	288	8.2	9.6	36.8	31.4	31.9	32.3
He + O ₂ ..	13.8	269	8.0	9.2	35.9	30.6	32.3	32.0
Difference and S. E.	$+1.5 \pm 0.8$	-19	-0.2	-0.4	-0.9 ± 0.6	-0.8 ± 0.5	$+0.4 \pm 0.1$	-0.3 ± 0.7
P value...	.1 - .2				.1 - .2	.1 - .2	.001 - .01	.6 - .7

¹ Volumes corrected to standard conditions: 0°C, 760 mm. Hg, dry. ² Volumes corrected to exhaled conditions: 32.2°C, 760 mm. Hg, 80% saturated. ³ These means are computed from the average values of 24 subjects.

had a higher exhaled vapor pressure at altitude, and one was unchanged. The mean decrease was corroborated by the vapor pressures determined from the dew points, which were consistently and significantly lower at altitude, as indicated by the small P value for the mean difference of -1.7 mm. Hg.

Density Changes. In an effort to isolate the influence of density alone, 8 subjects were tested at ground level, comparing breathing 100 per cent oxygen with breathing a lighter He-O₂ mixture. The absolute density of 23 per cent oxygen in 77 per cent helium approximates that of 100 per cent oxygen at 30,000 feet pressure-altitude. Breathing this gas mixture at ground level therefore simulated the decreased density found at 30,000 feet without the other physical and psychological effects of an altitude chamber 'flight.' To prevent possible bias in the results due to the influence of the first gas on the response to the second, the order of testing was alternated.

The results are shown in table 2. The calculated mean vapor pressures were

not significantly altered when breathing the lighter gas mixture. Six of 8 subjects increased their respiratory rate when breathing He-O₂, in agreement with the results of Specht (13), but the group increase was not significant. Dew point determinations of 24 subjects tested under the same conditions showed a small but statistically significant increase of 0.4 mm. Hg. The dew point was more difficult to read when the subject was breathing the lighter mixture, due to wider fluctuations between inspiration and expiration.

Temperature Changes. Thermocouple measurements at the nostrils of exhaled gas temperatures at ground level and altitude are included in table 1, and when

TABLE 3. CORRELATION COEFFICIENTS CALCULATED FROM RESPIRATORY DATA OF TABLE 1
All vapor pressures and volumes based on STPD corrections, 15 subjects at rest breathing oxygen

VARIABLES	r	P
<i>Ground Level</i>		
Rate of H ₂ O loss		
Minute Vol.....	+.81	<.001
Tidal Vol.....	+.46	.05 -.1
Resp. rate.....	+.29	.2 -.3
Vapor Press.....	+.42	.1 -.2
Vapor pressure, calculated		
Minute Vol.....	-.18	.5 -.6
Tidal Vol.....	+.30	.2 -.3
Resp. rate.....	-.42	.1 -.2
<i>Differences between Ground and Altitude Values</i>		
Rate of H ₂ O loss		
Minute Vol.....	+.69	.001-.01
Tidal Vol.....	+.38	.1 -.2
Resp. rate.....	-.09	.7 -.8
Vapor Press.....	+.52	.02 -.05
Vapor pressure, calculated		
Minute Vol.....	-.13	.6 -.7
Tidal Vol.....	+.35	.1 -.2
Resp. rate.....	-.46	.05 -.1

breathing oxygen and He-O₂ in table 2. They show no significant difference under our experimental conditions. The mean value of 32.2°C. used in the exhaled volume corrections is approximately one degree lower than that reported by Burch (14) for exhaled air.

Respiratory Rate Changes. Inspection of the individual results found at altitude suggested the possibility that changes in rate of respiration between ground and altitude might account for the alterations in water loss. In order to evaluate this possibility, correlation coefficients have been calculated from the data of table 1 and are presented in table 3. Two groups of correlations are shown. The first

indicates directly the relationship between water loss (expressed either as rate or as vapor pressure) and minute volume or its components, tidal volume and respiratory rate. Our data exhibit the usual high correlation found by others (6, 7) between rate of water loss and ventilation: $r = +.81$; $P = <.001$. Vapor pressure, however, does not correlate well with any of the other variables tested.

The second group of correlation coefficients was computed from the differences between ground level and altitude values. Again the rate of water loss is directly related to ventilation ($r = +.69$; $P = .001-.01$) and, in addition, it correlates significantly with the vapor pressure ($r = +.52$; $P = .02-.05$). In both the direct comparisons and the comparison of ground-altitude differences, the r values indicate that there is a greater probability (smaller P value) that vapor pressure correlates with respiratory rate than with either minute or tidal volume.

The effect of respiratory rate changes on the vapor pressure was tested experimentally. The 8 subjects breathing oxygen and He-O₂ were immediately retested while breathing each gas at approximately the same rate they had just used for the other gas. No clear-cut correlation was found between voluntary respiratory rate changes and vapor pressures when breathing either gas. These results were contrary to expectation and may be due to the fact that the change in respiratory rate was too small to be effective.

DISCUSSION

Observations on insensible water loss in the mountains and in a decompression chamber were made 45 years ago by Foa (15), who concluded that both lung and skin water loss are decreased at low barometric pressures. Guillemard and Moog (5) came to the same conclusions from mountain experiments. Schrotter and Zuntz, quoted by Guillemard and Moog, reported this effect in two balloon ascensions. These early reports were based on results obtained under uncontrolled experimental conditions of variable inspired temperature and humidity, using only a few subjects.

The effect of reduced barometric pressure on the physical characteristics of gases is undoubtedly a factor influencing lung evaporation. At altitude the decreased heat capacity of gas within the alveolar space would take proportionately less heat from the surrounding tissues so that the tissue fluid would be somewhat warmer and would be expected to induce a higher vapor tension than at ground level. At the same time heat conductivity of the gas is reduced, and warming of the alveolar gas to a given temperature would be somewhat delayed. Our failure to find differences in exhaled gas temperatures between ground and altitude seems to favor the effectiveness of factors influencing time of equilibrium attainment. In the case of He-O₂ inhalation, which has a greater heat conductivity than does O₂, there was also no demonstrable difference in exhaled gas temperature. The dew point measurements of aqueous vapor tensions at altitude and when breathing He-O₂ further substantiate this hypothesis. However, interpretations of actual experimental results in terms of physical variants are unwarranted because of the concomitant physiological changes.

The aqueous vapor pressure calculated from the gravimetric data represents the mean of several partial pressures, those of the various portions of the dead space

gas as well as that of the alveolar gas. The value of this average depends upon the magnitude of the individual partial pressures, and the ratio of dead space volume to alveolar volume. The exhaled vapor pressures calculated from the gravimetric data were usually found to be smaller than those calculated from the dew point data. This was to be expected, since the recorded dew point was the highest observed during exhalation, and thus would approach the maximum to be found in the breath. This discrepancy between the two values for exhaled air vapor pressures indicates that the dead space gas and probably some of the alveolar gas is at a lower vapor tension than the maximum measured by the dew point. The hypothetical aqueous vapor pressure of the admixed dead space gas whose volume has been computed from the mean ground level values of table 1 would be 24.6 mm. Hg in order to arrive at the mean value of 28.4 mm. Hg that we found. This computation assumes that the dew point figure represents accurately the vapor pressure of all the alveolar gas contained in the exhalation. This is probably not the case because of technical limitations and non-homogeneity of the gas from the alveolar spaces. It seems reasonable that the dead space gas would have a lower aqueous partial pressure, since at least some portion of it is the last gas to be inspired and the first to be exhaled, and so has a shorter exposure to evaporative surfaces, as well as a smaller surface-volume ratio than that of the deeper lung gases.

When our values from table 1 for ground level respiration are expressed as Burch (16) does his, we find a mean water loss from the lungs of 1.330 gm/10 min/m.² body surface, as compared to his figure of 0.878 gm. Since Burch's subjects were breathing room air, he deducted the inspired water content so that his value for water contributed by the subject is lower than ours. In our experiments, the total exhaled water represents an expenditure of energy by the body for evaporation from the respiratory surfaces. Benedict's subjects breathed dry oxygen, and our values are within the range that he reports (2).

From the results we had obtained previously (17) on 6 subjects exercising at ground level and simulated altitude, it was impossible to draw a conclusion regarding the effect of altitude on the output of respired water vapor. Present data obtained on resting subjects indicate that each dry liter of gas carries away from the lungs slightly more moisture at simulated altitude than at ground level. In terms of physiological volume, i.e. wet, there is, by contrast, less moisture per unit volume at altitude than at ground level in the exhaled air. This is confirmed by vapor pressures calculated from the dew point data independently by volume measurements which have been pointed out as suspect because they involve an arbitrary assumption regarding the very factor being examined.

Calculations of the correlation coefficients indicate that whereas water loss from the lungs per unit time is directly dependent upon the ventilation, the aqueous vapor pressure is more closely allied in an inverse manner with the rate of respiration than it is with tidal or minute volumes. Although this correlation is statistically not quite significant in our experiments, other investigators (7, 9) have shown that, at ground level, a rise in vapor pressure of exhaled air occurs with slowing of the rate, presumably because there is more time to approach saturation. Apparently, the slowing must be extreme to produce this result, or at least greater than our series

average. In individual cases where marked slowing occurred an increase in vapor pressure is apparent. This was most marked, for example, in *subject 18* (table 1), who slowed his respiration without great change in tidal volume, and increased the amount of water lost per minute from his lungs at altitude.

If we assume that rate is more important in determining the magnitude of the mean aqueous vapor pressure of an exhalation than is the tidal volume, which our data suggest, then we may suppose that the length of exposure to the evaporating surfaces is more critical than the amount of gas taken into the lungs. Even though the vapor pressures of alveolar and dead space gases differ, this difference must be relatively uncritical in determining the average vapor pressure of the total respiration since there is no demonstrable correlation with tidal volume. It would be interesting to determine whether or not the maximum vapor pressure, as measured by the dew point apparatus, is correlated with the tidal volume.

Since rate of breathing is partially effective in determining the water output from the lungs, we were interested in computing the hypothetical time required to reach an equilibrium value at reasonably high saturation as classically assumed in respiration literature. If the lung volume is assumed to be a layer spread uniformly over the total area of the lungs as a flat surface, a value for the thickness of this layer may be calculated: $2.75 \times 10^3 \text{ cm}^3 / 7.5 \times 10^5 \text{ cm}^2 = 3.66 \times 10^{-3} \text{ cm}$, or nearly 0.04 mm. This is a reasonable approximation of the radius of the alveolar passages. If we use this analogy for an approximation of the time required to saturate the inhaled volume, and with the further assumptions that the inhaled volume is instantaneously added, that complete mixing occurs, and that this volume represents a limiting case of the conditions set up for the usual diffusion formula given by Höber (18), we may compute an equilibrium time. Setting the concentration maximum at about 90 per cent, conservatively, to allow for the effects of reflection by a limiting barrier actually present in the tubular character of the lung surfaces, a maximum value for time of equilibrium of 1.16×10^{-3} seconds may be obtained. The order of magnitude of this value is so much smaller than that of the time values which are shown to be critical in saturation of the breath, as experimentally observed in our data, that a re-examination of the assumptions currently prevalent regarding the physical aspects of lung ventilation is indicated.

SUMMARY

The vapor pressures of exhaled gas have been calculated from gravimetric data and from dew point measurements. Fifteen subjects at rest breathing oxygen have been tested at ground level and at a pressure-altitude of 30,000 feet.

At altitude the decrease in gas volumes corrected to exhaled conditions was approximately the same as the decrease in rate of water loss, so that the computed vapor pressures did not significantly change from ground level to altitude. The concomitantly measured dew points of exhaled gas showed at altitude a statistically significant decrease in vapor pressure of almost 2 mm. Hg. When corrected to standard conditions (0°C., dry, ambient pressure), the respired volumes decreased proportionately more than did the water output, so that the calculated mean vapor pressure at altitude was approximately 3 mm. Hg higher than at ground level.

Correlation coefficients suggest that respiratory rate changes are more important in determining aqueous vapor pressures than are tidal and minute volumes.

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INFLUENCE OF DEAFFERENTATION ON STIMULATION OF MOTOR CORTEX¹

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GELLHORN'S studies of the effect of limb posture (1) and especially of fixation of joints (2) on the electromyographic (EMG) response to stimulation of the motor cortex reveal a considerable influence of proprioceptive reflexes on cortically induced movements. The magnitude of these effects of both passive stretch and active tension prompted an investigation into the question of the relative contribution to muscle response of proprioceptively induced reflexes and of the efferent impulses originating in motor cortex. The EMG and tension responses to cortical stimulation were therefore studied before and after section of the posterior roots to the limb.

METHODS

Cats were anesthetized with Dial-urethane (Ciba), 0.45 cc/kg. intraperitoneally and the motor cortex was exposed for stimulation with condenser discharges (Goodwin stimulator). The tibialis anticus and gastrocnemius muscles were used in all experiments. The copper wire recording electrodes were sewn about 1 cm. apart in the muscles for leading off Electromyograms (EMGs) which after suitable amplification were recorded with an Offner ink-writing crytograph. A torsion spring myograph was used for recording tension developed in tibialis (and in a few cases gastrocnemius) during isometric contractions. The spinal cord was exposed from L₄ to S₃, the dura reflected, and threads placed under the dorsal roots L₅ to SC for subsequent deafferentation by ligation.

RESULTS

Effects of Grading Cortical Stimulation. Slight increases in intensity or frequency of motor cortex stimulus result in increased amplitude of the EMG and in increased tension of the muscle by increasing the number and frequency of discharging units (3). These gradations in muscle responses are seen to persist after posterior root section, indicating that the motor cortex is not entirely dependent on proprioceptive recruitment for elicitation of graduated responses. A typical experiment illustrating the effect of slight variations in cortical stimulation on the response of tibialis before and after deafferentation is given in figure 1. The EMG developed in response to lower intensities of cortical stimulation (2.3-4.0 V) is similar before and after posterior root section with respect to threshold of response, summation time (decreased at 4.0 V and higher intensities) and amplitude.² Similar gradations

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² The differences in afterdischarge will be discussed elsewhere in conjunction with convulsive reactions.

of EMG response obtainable after deafferentation are shown in figure 3 in which the reactions of both tibialis and gastrocnemius are illustrated.

Experiments in which variations of frequency of cortical stimulation at a given intensity were studied also show that a graded EMG series can be obtained even after posterior root section. Frequencies from 20 to 90 per second were employed,



Fig. 1. TIBIALIS EMG in response to series of motor cortex stimuli at 90/sec. A-E controls; A'-F' after deafferentation. A, A' 2.3 volts, B, B' 3.3 V, C, C' 4.0 V, D, D' 4.8 V, E, E' 5.3 V, F' 6.3 V, vertical calibration 100 microvolts, horizontal calibration 1 sec. in all figures. There was no response at A and A'.

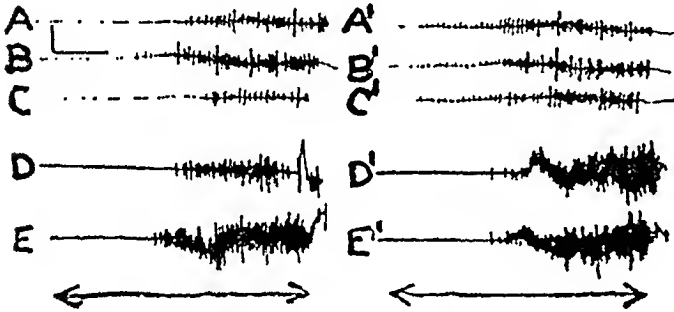


Fig. 2. EFFECT OF INITIAL LENGTH of tibialis on response to motor cortex stimulation before and after deafferentation. A-C, controls; A'-C' after deafferentation, 4.8 V, 90/sec. A, A', C, C' at resting length; B, B' 10 mm. beyond resting length. D-E, controls at 5.5 V, 73/sec. D'-E' after deafferentation, 8.0 V, 73/sec. D, D' resting length, E, E' stretched 10 mm.

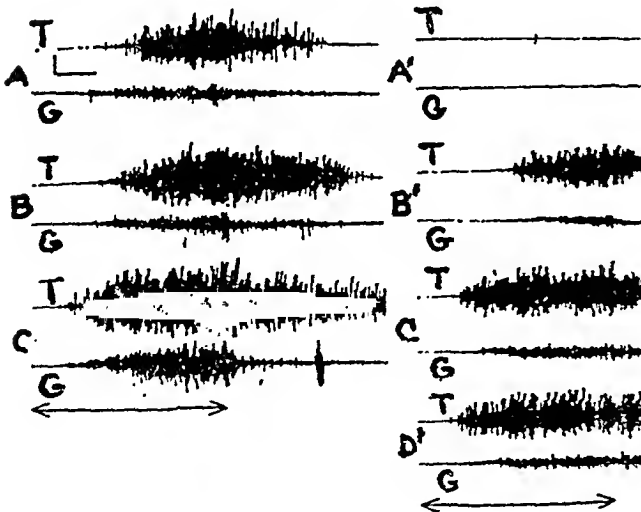


Fig. 3. CO-CONTRACTION IN GASTROCNEMIUS (G) with tibialis (T) response to motor cortex stimulation at 90/sec. A-C controls; A'-D' after deafferentation. A, A' 3.7 V, B, B' 4.0 V, C, C' 4.8 V, D' 5.5 V.

the voltage being kept constant unless threshold changes resulted from the deafferentation. That such threshold changes did not reflect injury to the cord is suggested by the fact that the threshold actually fell in some experiments though it rose in others. In one frequency series, e.g., 7.3 V was used in the control experiments, whereas after deafferentation 6.3 V gave a similar response.

Maximal Responses to Cortical Stimulation. With higher intensities of motor cortical stimulation, greater tensions and larger EMGs can be developed in the intact limb than are elicitable after deafferentation has eliminated proprioceptive recruitment. In table 1 are given figures for the tension developed by tibialis both before and after posterior root section; in each of the 4 experiments listed it can be seen that after its afferents had been severed the muscle failed to develop more than two thirds of the maximal control tension. In many of the experiments intensities of cortical stimulation higher than any used in the control series were tried after posterior root section, and even under the stronger cortical discharge resulting, the tension did not increase further.

That these tension differences are reflected (though in a less easily quantitated way) in the EMG can be seen in the experiments of figure 1 and figure 3. In the former, although as was seen earlier the threshold was unchanged by deafferentation

TABLE 1. TENSION DEVELOPED IN TIBIALIS IN RESPONSE TO VARYING INTENSITIES OF MOTOR CORTEX STIMULATION BEFORE AND AFTER POSTERIOR ROOT SECTION (L5-S3)

EXPT.	VOLTAGE	TENSION DEVELOPED, GM.		EXPT.	VOLTAGE	TENSION DEVELOPED, GM.	
		Control	Deafferented			Control	Deafferented
86R (fig. 1) Frequency 90/sec.	2.3	0	0	89R Frequency 80/sec.	3.6	52	0
	3.3	92	70		4.3	112	62
	4.0	140	140		5.3	132	92
	4.8	220	126		6.3	126	92
	5.5	260	116	86L Frequency 90/sec.	3.3	0	32
	6.3		120		4.0	160	50
85L (fig. 3) Frequency 90/sec.	3.7	336	90		4.8	170	80
	4.0	390	276		5.5	176	90
	4.8	470	270		6.3	182	104
	5.5		270		7.3	192	120
					8.0		116

and the responses at low cortical intensities were very similar with and without afferents intact, at higher voltages the EMG did not attain the amplitude after deafferentation which was seen in the control series. Figure 3 shows the same diminution in amplitude of EMG after posterior root section in response to higher intensities of motor cortical stimulation. The slight rise in threshold in this experiment (suggested by the similarity of response at 4.8 V after deafferentation and 3.7 V before) is not great enough to explain the small response of the deafferented muscle to 5.5 V cortical stimulation, a response which shows no increase over that obtained with 4.8 V.

It is thus seen that both EMG and developed tension show graduated responses to slight increases in intensity of motor cortex stimulation even after posterior root section, but that at higher intensities (where, in the limb with afferents intact, proprioceptive recruitment would play a rôle since considerable tension would be devel-

oped under the isometric conditions of the experiment) neither tension nor EMG attains the magnitude of the control responses at the corresponding voltages.

Effect of Varying Initial Length of Muscle. Increasing the initial length of a muscle (tibialis anticus) results in an increase in both EMG and tension developed in response to motor cortex stimulation (3). After posterior root section the EMG response is constant whether the muscle is stretched or slack, while the tension still increases with an increased initial length. In figure 2 are illustrated two such experiments in both of which the amplitude of EMG response when the tibialis is stretched beyond resting length is seen to be about twice that obtained when the muscle is initially at resting length (slack). In both experiments also the summation time is much shorter when the muscle is stretched, suggesting an interaction of proprioceptive impulses with impulses initiated by cortical stimulation. In the absence of proprioception, after deafferentation, the EMG response to cortical stimulation is seen to be independent of the initial length of the muscle with respect to summation time as well as amplitude, although the average summation time is reduced after deafferentation. The tension is increased when the muscle is stretched

TABLE 2. TENSION DEVELOPED IN TIBIALIS AT DIFFERENT INITIAL LENGTHS IN RESPONSE TO STIMULATION OF MOTOR CORTX BEFORE AND AFTER POSTERIOR ROOT SECTION

EXPT.	INITIAL LENGTH	TENSION DEVELOPED, GM.	
		Control	Deafferented
85	Resting length	50	38
	Stretched (+10 mm)	180	140
	Resting length	10	32
82	Resting length	15	30
	Stretched (+10 mm)	160	100

(table 2) both before and after posterior root section. This latter result was to be expected from studies on the length-tension relation of isolated muscle.

These examples suggest that the increased EMG response to cortical stimulation with increased initial length is dependent on the proprioceptive recruitment of additional motor units since it is absent after deafferentation; whereas the increased tension developed is related more directly to the increased initial length as it obtains even in the absence of proprioceptive recruitment.

Co-contraction. Under conditions of strong cortical stimulation or proprioceptive facilitation where considerable activity is seen in an agonist muscle (e.g., tibialis) co-innervation (or co-contraction) appears in the antagonist (4). In many animals in which an EMG response appeared in the gastrocnemius in response to higher intensities of cortical stimulation of a site giving a good tibialis response, this response was greatly diminished or even absent after deafferentation. One such experiment is illustrated in figure 3 where after deafferentation the gastrocnemius response is much reduced in comparison with tibialis EMGs. (Compare, e.g., control at 4.0 V and deafferentation at 5.5 V.) In several cats tension of gastrocnemius was re-

corded; in each of these cases, while stronger cortical stimulation resulted in development of small but measurable tensions in controls, after deafferentation no detectable tension was produced by the gastrocnemius even in response to quite high intensities of cortical stimulation. These experiments suggest that co-contraction is in many cases chiefly of proprioceptive origin. In a few cases the response in gastrocnemius was as large after deafferentation as before; presumably here the cortical site stimulated was one which excited the gastrocnemius itself sufficiently to cause it to respond even in the absence of proprioceptive reflexes from the tibialis.

DISCUSSION

The study of the action of proprioceptive impulses on spinal reflexes of Cooper and Creed (5) has been extended by Gellhorn (1) to a study of the effect of proprioception on cortically induced movements. In the present paper an attempt is made to separate the proprioceptive component of a muscle response from that due to impulses from the motor cortex. It has been seen that removal of proprioceptive impulses by appropriate posterior root section alters muscle response to motor cortex stimulation in the following ways: 1. While at low intensities of cortical stimulation a graded response is noted in both EMG and developed tension parallel to that obtained before deafferentation, at high cortical intensities neither EMG nor developed tension attains the amplitude seen with afferents intact. Apparently increase in the number of discharging motor units and increase in the rate of discharge result from stimulation of the motor cortex with increased intensity or frequency even after deafferentation. However, the maximal discharge attainable is reduced by the lack of proprioceptive recruitment. 2. Increasing the initial length of the muscle (which normally results in an increased EMG and tension response to a given cortical stimulus) after deafferentation is without effect on EMG, although still eliciting an increased tension. These experiments confirm, under the conditions of deafferentation, the conclusion drawn in an earlier work (3) that the amplitude of the EMG is an indication of the size of the central discharge. 3. Co-contraction as indicated by EMG is more markedly diminished by posterior root section than is the response of the agonist. 4. The effect of deafferentation upon the threshold response to cortical stimulation is minimal and variable as observed also by Sherrington (6).

Cutaneous impulses are of minor importance in these responses, as indicated by the fact that fixation of the limb prevented appreciable stimulation of cutaneous receptors during the experiments, and that the same effects could be obtained after cutaneous innervation had been removed by separating the skin of the leg from the underlying tissue. The effects of deafferentation enumerated above are therefore assumed to be due to the loss of proprioception.

SUMMARY

Slight variations in intensity and frequency of motor cortex stimulation result in graded responses of skeletal muscles as indicated by the magnitude of EMG and developed tension in normal cats as well as after deafferentation, showing that the motor cortex is itself capable of eliciting graded responses independent of proprioceptive recruitment. However, after deafferentation even maximal cortical stimulation

never induced the degree of tension nor the amplitude of EMG seen in control conditions. An initial stretch of a muscle causes an increased EMG and tension developed in response to motor cortical stimulation compared to that developed with the muscle slack under control conditions; after deafferentation the EMG response is seen to be independent of initial length of the muscle whereas tension remains directly related to initial length. Deafferentation markedly diminishes a co-contraction seen in gastrocnemius in most cases, suggesting a largely proprioceptive origin for co-contraction.

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CHARACTERISTICS OF TISSUE IMPEDANCE IN THE RAT UNDER CONDITIONS OF ELECTROCONVULSIVE SHOCK STIMULATION¹

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ATTEMPTS to control the intensity of the electrical impulse during electroconvulsive shock therapy have led to investigations of the nature of and rôle played by the impedance of the tissues through which the impulse flows. Some of these investigations (1) have indicated that d.c. resistance measurements are of little value in indicating true magnitudes of tissue impedance. Other studies (1-4) have employed low-intensity, high-frequency currents and have shown that, on application of the current, initial impedance drops instantaneously to a terminal value which is maintained throughout the remainder of the period of stimulation. The possibility has been suggested (5) that determinations of impedance employing low-intensity currents give high values which are not correlated with the resistance actually offered to the large shock currents necessary to induce convulsions.

The present studies were designed to clarify two aspects of this impedance phenomenon when observed during passage of the electroconvulsive shock current: 1) the relationships between the magnitude of the tissue impedance and the two variables, intensity of the electroconvulsive shock impulse and number of convulsive treatments, and 2) the relative significance of the resistive and reactive components contributing to the impedance.

METHODS

Two problems in measurement were presented. The magnitude of tissue impedance under operating electroconvulsive shock conditions had to be determined for a large number of subjects. This impedance had to be resolved into its resistive and reactive components, also under operating electroconvulsive shock conditions.

Determination of Magnitude of Tissue Impedance. The magnitude of the impedance was measured by the usual ammeter-voltmeter method. A known preset current delivered for constant periods of one second was supplied by the Pittsburgh Electroshock Unit (6); thus the current metering presented no particular problem. The voltage determination required a properly damped a.c. meter which would draw a negligible part of the total subject current. A Junior Volt-Ohmyst, used on the one hundred volt a.c. scale, satisfied these requirements.

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Knowing the current and voltage, it was possible to determine the impedance by employing the relationship, familiar in research on a.c. circuits, $Z = E/I$, where Z may be a complex number, i.e., $R \pm jX$.

In order to determine what relationships, if any, existed between impedance and current intensity of the electroshock impulse and between impedance and the number of convulsions induced, two groups, each containing 25 animals, were subjected to a series of electroshock convulsions. Each animal was convulsed on 5 consecutive days, an interval of 24 hours between convulsions being held as constant as possible for each animal. Each group of animals was divided into 5 equal sub-groups. Each sub-group was convulsed with a different current intensity applied for a duration of one second. These intensities ranged from 15 to 35 ma. in steps of 5 ma. This range was selected on the basis of previous research which had indicated that, at the duration employed, sub-convulsive behavior appeared with current intensities of less

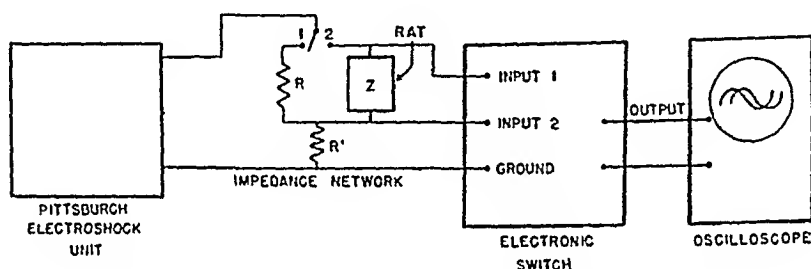


Fig. 1. BLOCK DIAGRAM showing the arrangement of equipment.

than 15 ma. and the incidence of paralysis increased very significantly above 35 ma. Observations of the two groups of subjects were made independently of each other, the second series taking place several months following the first.

The subjects were 50 male albino rats from the highly-inbred Sprague-Dawley colony and were all of approximately the same age and weight at the beginning of the research. The standardized procedure for controlling relevant variables while inducing the convulsions has been described by Russell *et al.* (6).

Analysis of Components of Tissue Impedance. The next problem was to determine the reactive component of this impedance, Z , under shock conditions using the 60-cycle source frequency. The circuit employed is shown in figure 1. This circuit makes use of the well-established fact that, in an a.c. circuit, current will lead the voltage if a capacitive reactance is present and will lag voltage if an inductive reactance is present. No lead or lag will occur if only pure resistance is present.

The important part of this system is the electronic switch. This instrument is composed of two separate square-wave generators with a common frequency control and synchronizer, and is capable of simultaneously reproducing two separate sine waves having a common reference point on an oscilloscope screen. A voltage wave proportional to and in phase with the current through Z may be obtained across a small noninductive resistor R' . The value of R' must be much lower than Z in order that the angle θ or phase shift angle will not be affected. The value of R' in this circuit was five ohms. The voltage wave was obtained across $Z + R'$. Using this apparatus arrangement, the minimum perceptible angle θ was determined em-

pirically to be slightly less than two degrees. During the experimental observations the subjects were inserted in the circuit in place of the test impedance Z .

This procedure for observing the relative contributions of resistive and reactive components to the total impedance was applied to each of a group of 37 male albino rats selected from the same colony and age group as those used in the preceding phase of the research. The same standardized procedure was followed for inducing the convulsions. A constant current of 20 ma. was used during all observations.

TABLE 1. RELATIONSHIPS BETWEEN IMPEDANCE AND CURRENT INTENSITY FOR EACH OF FIVE SHOCK TREATMENTS

TREATMENT	REPLICATION	ϵ^2	ϵ
1	1	.494	.703
	2	.544	.738
2	1	.474	.688
	2	.523	.723
3	1	.481	.693
	2	.659	.812
4	1	.404	.636
	2	.265	.515
5	1	.384	.619
	2		

An ϵ^2 of .364 is demanded at the 1 per cent level of confidence.

Because of the very significant rôle which the observer assumed in this procedure 4 observers simultaneously made independent observations of the wave forms appearing on the oscilloscope during the administration of the electroshock impulse. There was complete agreement on the results of each of these observations.

RESULTS

Initial Drop in Impedance. Observations of the magnitudes of tissue impedance during the passage of the electroconvulsive shock current provided evidence in support of the finding of other workers (3, 4) that an initial drop in impedance occurs on application of the stimulating current. This drop was revealed directly in a change of voltmeter reading which always occurred at the beginning of the current application. The inertia of the voltmeter needle made observations of the magnitudes of these drops impossible since the rising course of the needle occupied the time of some portion of the impedance drop. However, the voltmeter needle in all cases reached a peak of its rising course and then dropped to a steady level consistent with the terminal magnitude of the impedance during the passage of the convulsing current, indicating that an appreciable amount of time was required for the impedance to drop from its initial value.

Relationship between Impedance and Intensity of Electroconvulsive Shock Impulse. The relationships between the magnitude of impedance and the intensity of the electroconvulsive shock impulse were calculated for each of the treatments and are expressed in terms of the correlation ratio ϵ (7). Table 1 presents these ratios for each of the two experimental replications. The correlation ratios range in magnitude

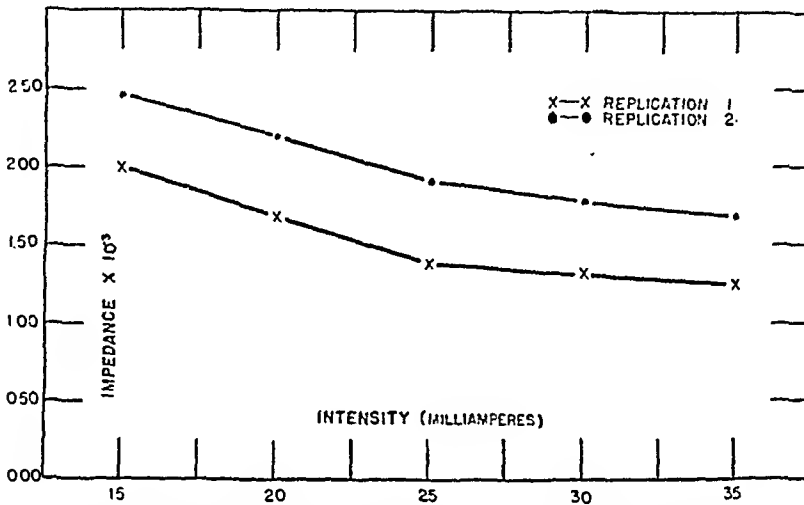


Fig. 2. RELATIONSHIP between impedance and current intensity.

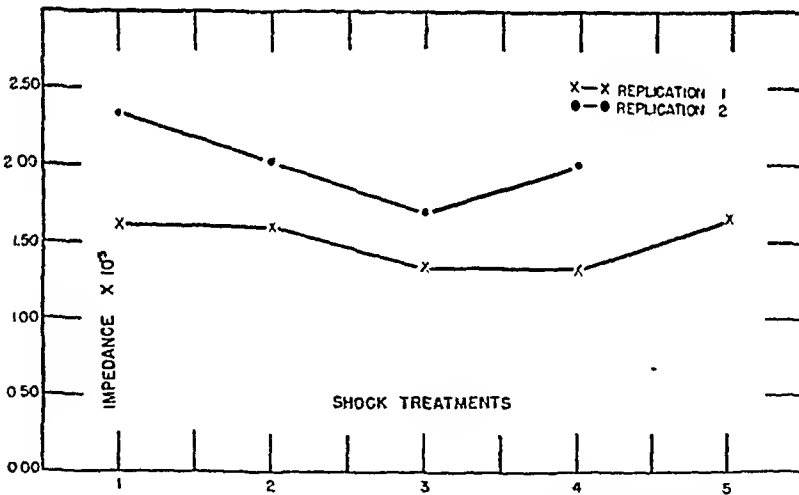


Fig. 3. RELATIONSHIP between impedance and number of shock treatments.

from .515 to .812 and indicate the existence of a very substantial relationship between the variables correlated.

Figure 2 represents this relationship graphically. It will be noted that the magnitude of the impedance diminishes systematically as the intensity of the electroconvulsive shock impulse increases. An indication of the confidence which can be placed in the significance of this relationship can be obtained from an examination of the ϵ^2 column in table 1. Under the conditions of the present study an ϵ^2 of .364 could be expected to occur by chance one per cent of the time (8). Since the observed values in all cases but one (replication 2, treatment 4) exceed the value of ϵ^2 at the

one-per cent point, the conclusion may be drawn with a high degree of confidence that the obtained correlations represent the existence of a true relationship. The displacement of the curves for the two replications may be accounted for in terms of the variation introduced by the use of different measuring instruments in the replications. In any event, it is the similar shape of the curves which is of importance in the present study.

Relationship between Impedance and Number of Electroshock Convulsions. The relationship between impedance and the number of electroshock convulsions is shown in figure 3. The correlation ratios (r) for the first and second replications are .342 and .424, respectively. The magnitudes of these ratios suggest definite though small relationships between the two variables, since they could be expected to occur by chance less than one per cent of the time.

Components of Impedance under Operating Electroconvulsive Shock Conditions. No phase shift between the voltage across and the current through the rat could be detected in any of the observations made. Since the minimum detectable phase shift angle was known to be slightly less than two degrees, this means that more than 99 per cent of the effective impedance of the rat measured under operating electroconvulsive shock conditions is pure resistance.

SUMMARY

The effective impedance of tissues to the flow of electroconvulsive shock impulses shows the following characteristics: A very substantial relationship, in the direction of decreasing impedance with increasing impulse intensity, exists between the magnitude of the impedance and the intensity of the impulse; a definite but small curvilinear relationship exists between the magnitude of the impedance and the number of stimulations, impedance decreasing initially and then increasing as the number of stimulations increase; and effective tissue impedance under operating conditions of electroconvulsive shock stimulation consists almost entirely of pure resistance.

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RELATION BETWEEN BRAIN AND PLASMA ELECTROLYTES AND ELECTROSHOCK SEIZURE THRESHOLDS IN ADRENALECTOMIZED RATS¹

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THE favorable effect of desoxycorticosterone therapy in certain cases of epilepsy, described by McQuarrie, Anderson, and Ziegler (1, 2), suggests that alterations in electrolyte balance may be a factor in precipitating grand mal seizures. Evidence that brain excitability may be increased by extracellular electrolyte depletion has been presented by Swinyard (3), who found a positive correlation between extracellular sodium concentration and electroshock seizure threshold in experimentally hydrated rats. In normal rats, Woodbury and Davenport (4) observed an elevation in electroshock threshold after the intraperitoneal injection of isotonic solutions of NaCl or CaCl₂ or after chronic treatment with desoxycorticosterone; conversely, a fall in threshold occurred after the intraperitoneal injection of isotonic solutions of KCl or MgCl₂. Since the adrenalectomized rat not given supportive treatment is known to show changes in plasma and muscle electrolyte concentrations (5-6), the work presented here was undertaken to investigate the relationship of electrolyte alterations in adrenalectomized rats to cerebral excitability as measured by seizure threshold. |

METHODS

Sprague-Dawley male rats weighing approximately 160 gm. were adrenalectomized and were allowed to drink 0.9 per cent NaCl solution until they weighed at least 200 gm. Only those rats which responded by a prompt loss of weight to deprivation of this source of extra Na were used, after a recovery period on 0.9 per cent NaCl solution, for the studies reported here. Normal rats of the same age as the adrenalectomized ones were used as controls for electrolyte analyses. All were given free access at all times to a stock diet consisting of a commercial calf meal² to which were added 3 per cent brewer's yeast and 3 per cent wheat germ.

The salts listed in tables 1 and 2 were administered to the adrenalectomized rats by allowing the animals to drink the appropriate isotonic solution *ad libitum* for the number of days indicated. A single dose of desoxycorticosterone acetate³ (DCA) in 1 ml. of sesame oil was given by subcutaneous injection. This dose of sesame oil itself has no effect upon the electroshock threshold.

The electroshock seizure thresholds were determined by a method already described (8). An interval of 4 hours or longer was allowed between shocks, and whenever possible the rats were not shocked on more than 3 consecutive days. The threshold is the smallest amount of current which,

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² Known as 'Calf Builder' and prepared by General Mills, Inc., Minneapolis, Minn.

³ The desoxycorticosterone acetate was kindly supplied by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc.

applied for 0.2 second, produces a detectable clonic seizure. Its value, expressed in milliamperes (mA) is nearly constant from day to day but increases slowly with age (9).

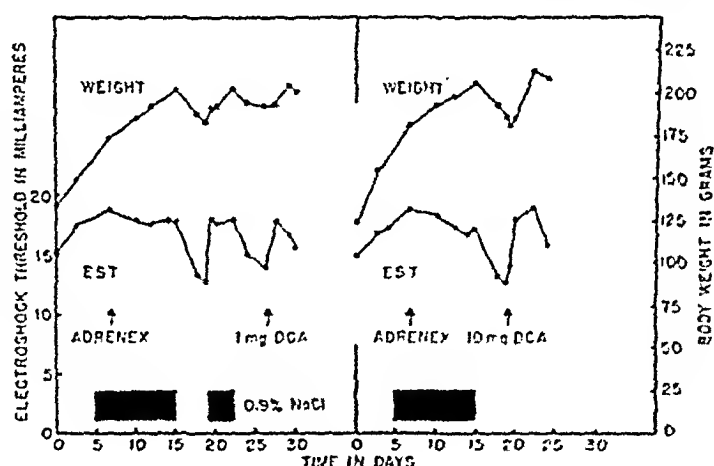
Blood for chemical analysis was obtained by heart puncture of the unanesthetized rats, using heparin as an anticoagulant. The animals were then decapitated, the brains removed, and the cerebral cortices separated from the major portion of the white matter. Blood or brain samples of 3 to 6 rats were pooled. Plasma was separated under oil. Water content was determined by drying the pooled brain samples and weighed aliquots of plasma to constant weight at 105°C. Chloride was determined in plasma by the method of Van Slyke and Sendroy (10) and in brain by the same method, using the Sunderman and Williams (11) technique of alkaline digestion. Plasma was analyzed for calcium by the Clark and Collip (12) modification of the method of Kramer and Tisdall (13). A slightly modified Perkin-Elmer flame photometer (Model 52A) was used for the determination of sodium and potassium in ashed brain and plasma. The material was prepared for analysis by the method of Hald (14).

Blood for sugar determination was freshly drawn by cardiac puncture and analyzed by the method of Hagedorn and Jensen (15).

RESULTS

A preliminary experiment, the results of which are shown in figure 1, suggested that the electroshock seizure threshold of the rat may be correlated with the electro-

Fig. 1. Upper curves: average body weight in grams. Middle curves: average electroshock threshold (EST) in milliamperes. Bottom bars: presence of 0.9% NaCl in drinking water.



lyte balance. Adrenalectomized rats maintained on 0.9 per cent NaCl solution showed only slightly lower thresholds than before operation. Removal of NaCl from the drinking water resulted in an abrupt drop in threshold. After 4 days, when the threshold had fallen by at least 20 per cent, the restoration of 0.9 per cent NaCl solution or the injection of DCA brought the threshold back to the control level within 24 hours. In fact, the larger dose of DCA (10 mg/rat) raised it slightly but significantly above the control level for about 48 hours. This experiment was repeated on 10 larger rats with similar results.

Table 1 summarizes the electroshock threshold changes which resulted when adrenalectomized rats were deprived of 0.9 per cent NaCl solution and were given water or other salt solutions to drink. In all cases there was a mean weight loss of 8 to 10 gm/rat/day. There was a sharp drop in threshold when water was substituted for NaCl solution, and a somewhat more abrupt fall when isotonic KCl or MgCl₂ solution was substituted. Replacement of NaCl by isotonic CaCl₂ solution had little effect on the threshold after the first day, in spite of the steady weight loss of the animals. The percentage of rats surviving after 4 days on CaCl₂ was greater

than that surviving after 4 days on water, 3 days on $MgCl_2$ or 2 days on KCl solution. In all cases there was a prompt return of the electroshock threshold to its original level or higher when NaCl was restored. This original level was approximately the same as that of normal rats of the same age.

Table 2 shows the results of analyses of plasma and brain cortex for water and electrolytes. In no case was the plasma or brain water content significantly different from normal. The brains of adrenalectomized rats, comparable to those of animals showing the marked electroshock threshold changes indicated in table 1, were normal with respect to Cl, Na, and K concentrations. The only significant change in plasma

TABLE 1. ELECTROSHOCK THRESHOLDS OF ADRENALECTOMIZED RATS

NaCl CONTROL PERIOD		NaCl WITHDRAWAL PERIOD				NaCl RECOVERY PERIOD, 24 HRS.	
No. of rats	EST† ma.	Treat- ment	Days	No. sur- viving	EST ma.	No. sur- viving	EST ma.
13	18.4 ± 0.5	H_2O	1	13		9	18.8 ± 0.4
			2	13	* 15.2 ± 0.5		
			3	12			
			4	11	* 14.1 ± 0.4		
11	20.5 ± 0.2	$CaCl_2$	1	11	* 18.8 ± 0.3	10	20.6 ± 0.4
			2	11	19.9 ± 0.2		
			3	10			
			4	10	20.4 ± 0.4		
13	18.4 ± 0.4	$MgCl_2$	1	13		8	19.1 ± 0.4
			2	13	* 14.7 ± 0.5		
			3	8	* 13.5 ± 0.8		
13	20.0 ± 0.5	KCl	1	13	* 15.4 ± 0.7	8	20.4 ± 0.5
			2	9	* 14.7 ± 0.5		

* Significantly different from control value. $P = 0.01$ or less.

† EST = electroshock threshold.

chloride was the lower than normal concentration in the adrenalectomized rats after 4 days on plain water. Plasma K was elevated by 31 per cent in adrenalectomized rats on NaCl solution and by 67 to 75 per cent in the other cases. Plasma Na concentration was normal in adrenalectomized rats maintained on NaCl solution. It was depressed to a highly significant extent in adrenalectomized rats on water, KCl or $MgCl_2$ solution, but was lowered in the rats on $CaCl_2$ only enough to be statistically significant. The plasma Na concentration of the adrenalectomized rats on $CaCl_2$ was significantly higher than that of similar animals on water.

When the adrenalectomized rats were deprived of the supportive effect of extra NaCl they showed the expected progressive loss of appetite. This was reflected in

TABLE 2. WATER AND ELECTROLYTE CONCENTRATIONS PER KILOGRAM OF WET BRAIN OR PLASMA IN NORMAL AND ADRENALCTOMIZED RATS

	N	PLASMA					BRAIN				
		H ₂ O	Cl	Na	K	Ca	H ₂ O	Cl	Na	K	
		gm.	mEq.	mEq.	mEq.	mEq.	gm.	mEq.	mEq.	mEq.	
Normal controls	10	921±2	107.1±0.6	142.0±0.3	4.8±0.1	5.5±0.1	794±1	34.2±0.1	50.0±0.2	101.6±0.7	
Adrenex. NaCl	8	925±2 (0.2)	106.4±0.9 (0.5)	141.2±0.6 (0.2)	6.3±0.3 (0.001)	5.6±0.1 (0.3)	795±1 (0.6)	34.4±0.1 (0.3)	50.3±0.4 (0.6)	102.1±0.9 (0.6)	
Adrenex. CaCl ₂ , 4 days	9	920±1 (0.7)	107.3±0.5 (0.7)	139.3±0.6 (0.01)	8.0±0.2 (0.001)	5.9±0.2 (0.8)	792±1 (0.5)	34.9±0.4 (0.1)	50.1±0.4 (0.7)	102.8±1.0 (0.3)	
Adrenex. H ₂ O, 4 days	3	915±2 (0.1)	101.0±1.3 (0.001)	129.7±1.5 (0.001)	8.1±0.3 (0.001)	4.7±0.5 (0.05)	793±1 (0.7)	34.8±0 (0.3)	49.8±0.3 (0.6)	101.6±1.1 (0.9)	
Adrenex. MgCl ₂ , 3 days	2	915±2 (0.2)	107.3±0.3 (0.8)	129.5±2.3 (0.001)	8.3±0.8 (0.001)	5.7±0.4 (0.3)	791±1 (0.4)	34.7±0.5 (0.5)	51.1±0.4 (0.8)	101.9±0.6 (0.8)	
Adrenex. KCl, 2 days	2	917±2 (0.2)	106.8±0.2 (0.8)	131.0±0 (0.001)	8.4±0.1 (0.001)	6.0±1.4 (0.7)	792±1 (0.5)	34.1±0.1 (0.4)	50.5±0.2 (0.3)	100.7±0.2 (0.5)	

N = number of pooled samples. Mean number of rats per pool = 4.

Figures in parentheses are P values of differences from controls.

the blood sugar levels. As compared with a control (NaCl) blood sugar level of 118 mg. per cent, 16 adrenalectomized rats after 4 days on CaCl_2 had a mean level of 88 mg. per cent and 6 adrenalectomized rats after 4 days on water had a mean level of 92 mg. per cent.

DISCUSSION

The brain of the adrenalectomized rat appears to have a striking ability to preserve its normal content of Na and K under conditions which result in marked changes in the plasma levels of these cations. The apparent disagreement of this finding with a recent report by Hoagland and Stone (16) of increased brain K in adrenalectomized rats may probably be explained on the basis of technique. These investigators found a greater intensity of radioactivity in the brains of adrenalectomized rats than in those of normal rats 36 hours after the injection of radioactive potassium, K^{42} . Woodbury (17) has found that 48 hours after the injection of K^{42} the normal rat brain is continuing to take up K^{42} from the plasma. The results obtained by Hoagland and Stone may therefore indicate a more rapid turnover of K rather than an increase in total brain K in the adrenalectomized rat. The data presented here show no change in brain Na when the plasma Na has fallen by about 9 per cent, and no change in brain K when the plasma concentration has risen by 75 per cent. In this respect, the brain of adrenalectomized rats is comparable to the liver tissue (7) and contrasts markedly with skeletal muscle (5-7) which manifests electrolyte changes similar to those of plasma. Woodbury and Davenport (4) have found a similar constancy of rat brain electrolyte under conditions of increased plasma Na and low plasma K concentrations.

It is apparent that changes in the electroshock seizure threshold observed in adrenalectomized rats are not the result of changes in brain water, Cl, Na, or K concentrations, nor are they correlated with any change in plasma water, Cl, or Ca concentrations. The possibility that they are correlated with the blood sugar level is excluded by the finding of similar blood sugar concentrations in adrenalectomized rats after 4 days on water, when the threshold was lowered by 23 per cent, and in those after 4 days on CaCl_2 , when the threshold was normal. The elevated plasma K in all the adrenalectomized rats, especially in those rats on CaCl_2 , indicates that changes in the plasma level of this ion exerted no effect on the electroshock threshold. There is, however, a correlation between plasma Na level and electroshock threshold. Apparently, if the plasma Na concentration falls below about 139 mEq/l. there is a corresponding drop in threshold, indicating an increased excitability of the central nervous system.

SUMMARY

Adrenalectomized rats maintained on 0.9 per cent NaCl solution have approximately normal electroshock seizure thresholds. On plain water they show a prompt fall in electroshock threshold which is quickly reversed by replacement of NaCl or by the injection of desoxycorticosterone. Substitution of isotonic KCl or MgCl_2 solution for NaCl solution results in an abrupt fall in electroshock threshold, but on isotonic CaCl_2 the adrenalectomized rats are able to maintain normal brain excita-

bility for 4 days. In all these circumstances, the adrenalectomized rats have normal brain concentrations of water, Cl, Na, and K.

The blood plasma of adrenalectomized rats contains normal concentrations of water and Ca and increased concentrations of K in all cases. The plasma Cl concentration is normal in all except the animals drinking plain water. The plasma Na concentration is normal in adrenalectomized rats maintained on NaCl solution, slightly decreased in rats on isotonic CaCl_2 and decreased to a highly significant extent in rats on water or KCl or MgCl_2 solutions.

It is concluded that the electroshock seizure threshold of the adrenalectomized rat is directly correlated with the plasma sodium level.

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SURVIVAL TIME OF REFRIGERATED MAMMALIAN MUSCLE AND NERVE AFTER SOMATIC DEATH

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A PREVIOUS communication (1) reported that frog skeletal muscles, when kept immersed in a dextrose Ringer's solution near 0°C., preserved their excitability to direct faradic stimulation practically undiminished for an average of 11 days, and became inexcitable in 15 days on the average, though sometimes not till 29 days. Response to nerve stimulation also remained practically undiminished for an average of 7½ days, and was extinguished in 12½ days on the average, sometimes not till 17 days. Contralateral spinal reflexes began to weaken in 11 to 14 days and were abolished in 11 to 17 days.

It appeared interesting to determine the survival time of mammalian skeletal muscle under similar conditions, especially with a view to the possibility of the surgical re-implantation of refrigerated amputation tissue. Carrel (2) reported successful union of the amputated leg in the dog by immediate vessel suture, with survival of 8 months, as long as the animal was observed. Blackmore, Lord and Stefko (3) report successful re-implantation of the legs of two dogs, with non-suture arterial anastomosis, the amputated legs having been kept on cracked ice overnight. The photographs taken 12 and 21 days later show excellent union of the skin, but marked edema of the amputated legs. Dr. Blackmore informs us that the leg was paralyzed as the nerves were not sutured; and that the muscles were atrophic in the gross when the dogs were killed at 40 days. The experiments therefore do not show that the muscle and nerve survived so as to be capable of resuming function. The data which we report herewith appear to preclude this. Earlier experiments of Mangold (4) on mice and Winterstein (5) on rabbits also indicate that the excitability of mammalian skeletal muscle is completely abolished in 24 to 66 hours when the muscle is immersed in saline solution at 8 to 18°C.; within 8½ hours at 36 to 38°C. These reports do not describe the degree of response, but it is probable that decisive disorganization occurs much earlier, some fibers deteriorating more rapidly than others. This is revealed by the experiments here described.

METHODS

The experiments were made on young, well nourished white rats and mice. One of the rats (no. 5) was killed by crushing the cervical vertebrae. The others were injected intraperitoneally with Seconal sodium; one, (no. 6) with 30 mg/kg. of body weight, which produced deep basal anesthesia; three (nos. 7, 8 and 10) with

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60 mg/kg., producing full anesthesia. They were decapitated, giving mild or no convulsions, plunged into a bath of mammalian dextrose-Ringer's solution (NaCl 0.85, KCl 0.03, CaCl_2 0.025, MgCl_2 0.01, NaHCO_3 0.02, dextrose, 0.1 per cent), pre-refrigerated to near 0°C., and freely aerated. The carcass was immediately skinned, eviscerated, and dissected in the cold bath, to make preparations of the hindlegs, forelegs, flanks, ribs, tail and face. The preparations were suspended in a 4-liter bath so as to be freely surrounded by the refrigerated solution, which was kept aerated by a brisk stream of air. Their excitability was checked from time to time by direct stimulation of the exposed muscles with a Harvard inductorium activated by a $1\frac{1}{2}$ -volt dry cell, with the secondary coil set at 12, 6, and 0 cm. The following code was used in grading the responses: 'very good'—good response at 12 cm.; 'good'—good response requiring 6 cm.; 'fairly good'—good response requiring 0 cm.; 'fair'—fair response at 0 cm.; 'slight'—slight response at 0 cm.; 'inexcitable'—no response at 0 cm. The bath with the preparations was kept in the refrigerator between stimulations and overnight.

RESULTS ON RATS

These are described only in abstract. The results with the hindlegs may be taken as typical. In the rat without anesthesia (*no. 5*) the response to direct stimulation declined after decapitation to 'fairly good' within half an hour, to 'slight' during $1\frac{1}{2}$ to $4\frac{1}{2}$ hours. It was 'inexcitable' at 24 hours. In the four rats with Secional the response started to decline rapidly from 'very good' at 4 minutes, reaching 'good' in 5 to 15 min., median 10 min.; 'fairly good' in 20 min. to > 8 hrs., median 25 min.; 'fair' in 32 min. to > 8 hrs., median 5 hrs.; 'slight' in 32 min. to > 8 hrs., median 7 hrs.; 'inexcitable' in 1 to < 22 hrs., median > 8 < 22 hrs.

It appears therefore that the hindleg muscles of rats, kept under the best conditions, invariably become completely inexcitable in less than 24 hours, and sometimes as early as one hour, after removal from the body. The deterioration is generally quite marked within 25 minutes. Within these limitations there are marked differences between individual rats, but both hindlegs of a rat behave practically alike. There was nothing apparent in the experiments which would account for the differences.

The forelegs of the anesthetized rats deteriorated similarly to the hindlegs perhaps somewhat more slowly on the average. The median time when the response reached 'good' was 10 min.; 'fairly good' 6 hrs.; 'fair' 7 hrs.; 'slight' > 7 hrs.; inexcitable > 8 < 23 hrs. The difference could conceivably be due to different thickness of the tissue. This is somewhat doubtfully confirmed by the muscles of the flank: the median decline to 'good' was in 25 min.; 'fairly good' 3 hrs.; 'fair' 6 hrs.; 'slight' $8\frac{1}{2}$ hrs.; 'inexcitable' < 23 hrs. The median responses of the even thinner costal muscles reached 'good' in 10 min.; 'fairly good' in 2 hrs.; 'fair' in 6 hrs.; 'slight' in > 8 hrs.; 'inexcitable' in > 7 to < 24 hrs. Altogether, the differences do not progress convincingly with the thickness of the muscles from the ribs to the legs of the rat. In any case, they are less than the differences between the different animals. The diaphragm muscle also falls within the same range. In *rat 7* the response declined to 'fair' in 10 min. and it became 'inexcitable' in 45 min. In *rat 10* it was

still 'fairly good' in 7 hrs., 'inexcitable' in 22 hrs. The face muscles (eyelids and whiskers) deteriorated very early. In *rat 6* they were 'inexcitable' in 15 min.; in *rat 5*, within an hour. The tail muscles in *rat 8* gave 'fairly good' responses in $\frac{1}{2}$ to 7 hrs.; 'fair' in $8\frac{1}{2}$ hrs.; 'inexcitable' in 22 hrs.

The question whether the differences among individual animals extend to all muscles is answered by comparison of the 3 anesthetized rats in which four muscles were studied. There was general agreement in the deterioration of the hindlegs, forelegs and flank muscles, all declining most rapidly in *rat 8*, then *rat 10*, slowest in *rat 7*; but costal muscles deteriorated fastest in *rat 10*. It appears therefore that the differences in the individual animals extend to the various muscles, excepting the costal. We have not found an explanation why the individual animals should differ in their deterioration rate. All had received the same dose of Seconal and all were in deep narcosis. Convulsions were absent in *rats 7* and *10*, weak in *rat 8*. The time between the injection and decapitation was 9 minutes in *rats 7* and *8*, 11 minutes in *rat 10*.

Rigor mortis, which sets in so much earlier in mammals than in frogs, might conceivably be concerned in the difference between individual rats, as well as between rats and frogs. It would interfere more or less with the response, according to the proportion of muscle fibers that are rigid at the time. The experimental procedures were therefore planned to minimize rigor, by narcosis, rapid excision, cooling, aeration, and alkalization of the solution. In any case, the data show no relation between the rate of deterioration of excitability on the one hand, and development of rigor on the other: In *rat 8*, whose excitability deteriorated most rapidly, the leg muscles were semirigid in 20 minutes, but their response was still fairly good. They became inexcitable in an hour. In *rat 7*, with the slowest deterioration of excitability, the leg muscles were semirigid in 10 minutes, with good response at 25 minutes; and at 8 hours they were still semirigid with good response; at 24 hours they were very rigid but still gave a slight response. *Rat 10*, with intermediate deterioration rate, showed no apparent rigor in 5 hours, with fairly good response. *Rat 6* gave a fair response in 6 hours, when it was rigid.

Since Winterstein (5) showed that the addition of 0.1 per cent of hydrogen peroxide prevents rigor, one of the forelegs of *rat 6* with good response was transferred at the end of an hour to refrigerated Ringer's solution with the addition of hydrogen peroxide, without aeration; the other foreleg, with fairly good response, was left in the refrigerated solution without this addition. In two hours the response of the peroxide muscle had fallen to 'fair', while the control was still 'fairly good'. The control leg was rigid, the peroxide-treated leg was soft. In 20 hours both responded slightly, the peroxide leg still being soft, the control rigid. It appears therefore that the peroxide inhibited the development of rigor, but had no effect on the deterioration of excitability. Altogether, then, the rigor does not explain the variation between individual animals.

A somewhat unsuccessful attempt was made to render a rat 'cold blooded' by refrigeration before decapitation. It was narcotized with Seconal sodium, 40 mg/kg., and placed in a refrigerator near 0°C. However, it died in two hours. The unskinned carcass was immersed in refrigerated Ringer's solution for 22 hours. The muscles of the hindleg and flank were found inexcitable to direct

stimulation. Pre-refrigeration for two hours before death therefore did not succeed in delaying the loss of excitability.

The response to indirect (nerve) stimulation always declined much faster than the direct, and the difference was materially greater than for frogs. The time after decapitation when the decline in five preparations reached 'fairly good' ranged, for direct stimulation, from 20 mins. to 8 hrs., median 7 hrs.; for indirect stimulation, 20 min. to $2\frac{1}{2}$ hrs., median $1\frac{1}{2}$ hrs. The time for indirect averaged about 25 per cent of the direct with rats, 81 per cent with frogs. 'Inexcitable' was reached in 1 to > 8 hrs., median $> 7 < 22$ hrs.; for indirect stimulation in 20 min. to $7\frac{1}{4}$ hrs., median 4 hrs.; the indirect averaging about 33 per cent of the direct with rats, 83 per cent with frogs.

OBSERVATIONS ON MICE

A few experiments were made to see how the rate of deterioration of response to direct stimulation of the muscle compares in the two species, employing the same methods. The results are of the same order as in the rats and show similar differences between individual animals. The time required for the deterioration to reach 'fairly good' ranged from 25 min. to 6 hrs.; 'inexcitable,' 35 min. to 43 hrs.

COMMENTS

The early deterioration of the skeletal mammalian muscle remains unexplained. It is not due to the peculiar structural configuration of skeletal muscle, for it contrasts sharply with the much slower degeneration of frog muscle which has a similar structure. Nor does it extend to mammalian contractile tissues in general: It is known that the smooth muscle of mammalian intestines performs peristaltic contractions after being kept on ice for 5 days. Mammalian iris also reacts very satisfactorily at least 24 to 30 hours after excision, if the whole eye is kept in iced Locke solution. So also does the excised human uterus. Human spermatozoa may show normal motility when thawed after being frozen at -79°C . for 40 days, and some even after 70 days.

SUMMARY

The survival of the skeletal muscle of rats and mice after somatic death is very much shorter than with frogs. Notwithstanding all precautions to prolong survival, by barbiturate narcosis, immersion in ice-cold dextrose Ringer's solution, and ample aeration, the response to direct faradic stimulation declines generally within an hour, and becomes stabilized at a low level between 30 minutes and 8 hours, and is practically always completely lost overnight. Muscle rigor also occurs early, but this is not the cause of the loss of excitability, as muscle in rigor may still respond to stimulation. Response to stimulation of the nerve is abolished considerably before response to direct stimulation, so that the nerve fibers or endplates deteriorate even more rapidly than the muscle.

The rate of deterioration differs somewhat for individual muscles, but practically all lose their excitability completely within 24 hours. It appears therefore that

re-grafting of excised muscle would not be successful, except perhaps if it is done very promptly.

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LOSS OF EXCITABILITY OF FROG SCIATIC NERVE AND SKELETAL MUSCLE ON DEEP-FREEZING

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A PREVIOUS investigation (1) showed that frog nerve, skeletal muscle, and some spinal reflexes, preserve their excitability for 2 or 3 weeks when kept in a dextrose-Ringer's solution at near 0° C. It is known that exposure to temperatures of -1.6 to -3.0° C. generally destroys the response of muscle in a few hours; but if ice crystals do not form it may be subcooled to -18° C. and recover (2, pp. 54 and 85). The excitability of frog nerve does not recover if it is cooled below -1.8° C. Very rapid 'ultra-freezing' of tissues by immersion in liquid air or liquid nitrogen, or in isopentane cooled with liquid nitrogen, is generally less injurious than slow freezing, since solidifying of the water occurs without visible crystallization. Definite data as to the resistance of frog nerve to ultra-freezing appear to be lacking, but its excitability is presumably destroyed. Homoplastic mammalian nerve, frozen in nitrogen-chilled isopentane and dehydrated by high vacuum and phosphorus pentachloride and stored for several months at -40° C., is as suitable for nerve grafting as is living nerve (3, 4). Since heteroplastic nerve treated in the same manner behaves as a foreign body, the frozen nerve has preserved its species identity; but there is no evidence that it is functionally revivable. Frog gastrocnemius muscle does not survive freezing in liquid air (5); but if bundles of 8 to 10 muscle fibers are frozen in liquid air for a few seconds to several hours and then thawed, some fibers may contract to strong stimulation (6).

The injurious effects of exposure to low temperatures, as tabulated in the monograph of Luyet and Gehenio (2, pp. 237-250), vary materially for different tissues and organisms and reports of different observers do not agree. The injury depends to a great extent on the formation of ice crystals, which not only produce mechanical injury of the cells but which also disorganize the colloids. In any case, survival at low temperatures requires the absence of gross ice-crystal formation, which may be secured by different mechanisms in different cases. Low water content is one important factor. The speed of freezing also influences the formation and growth of ice crystals, the injury being least when the freezing is done most rapidly (7).

Most plants and animals die in the neighborhood of their freezing point, a degree or two below 0° C., at which time a large part of their water crystallizes as ice. Some forms, especially those containing little moisture, are not killed by ultra-freezing in liquid nitrogen. Among these are most bacteria, yeasts, and some flagellates (trypanosomes). Infusoria and spores (other than motile) remain viable even when plunged into liquid hydrogen at -252° C (8). All encysted forms and organisms which support desiccation survive indefinitely in liquid air. Rhizopods, ciliates and some flagellates are killed at a few degrees below 0° C. Paramecia and amebae, even under the best con-

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ditions, are always killed by immersion in liquid air. Ova of invertebrates often resist temperatures of -30° to $-40^{\circ}\text{C}.$, but the ova of vertebrates, including amphibians, are killed by those of a few degrees below $0^{\circ}\text{C}.$ (2, pp. 32, 43, 94).

Human spermatozoa are resistant to subfreezing temperatures. Many, but not all, of the cells, recover normal motility on thawing after freezing at $-79^{\circ}\text{C}.$ for 40 days (9) or 70 days (10), and even when frozen in ampules at $-196^{\circ}\text{C}.$ for 8 days and thawed rapidly at $37^{\circ}\text{C}.$ (11). It is not known whether or not ultra-frozen human spermatozoa are able to fertilize. However, while fowl sperms frozen at $-79^{\circ}\text{C}.$ for an hour were fertile, the embryos died very early (12). The spermatozoa of rats are reported to be killed by immersion in liquid air (2, p. 221).

Isolated tissues are generally killed by a few degrees below $0^{\circ}\text{C}.$ Plant epidermis, tissues of stems, roots and leaves, embryonic animal tissues, ciliated epithelium, muscular and apparently nerve tissues are among those thus affected. Partial dehydration increases the resistance of some materials (2, p. 60). Freezing in liquid air destroys the utilization of oxygen by rat heart, liver, kidney, lung, spleen and testes (13). Corneas of rabbits, frozen in liquid nitrogen, dehydrated in vacuum at $-40^{\circ}\text{C}.$ and rehydrated in Ringer's solution, failed to produce transparent grafts, although they healed in place (14, 15). Human skin grafts appear remarkably resistant. Preserved and frozen in plasma at -20° to $-25^{\circ}\text{C}.$ for 60 days, they give as many takes as fresh grafts (16). Frozen at $-70^{\circ}\text{C}.$ and lyophil dried, 80 per cent of the grafts survive, although they are less satisfactory than fresh skin (17).

Of multicellular plants, most fungi and algae are killed when exposed to -10° to $-15^{\circ}\text{C}.$, but some fungi withstand liquid gases. Most higher plants are killed by a few degrees below $0^{\circ}\text{C}.$, but some resist -30° or $-40^{\circ}\text{C}.$ (2, p. 70).

Of multicellular animals, invertebrates are generally killed when frozen somewhat below $0^{\circ}\text{C}.$ Some insect larvae support about $-20^{\circ}\text{C}.$, but die below this temperature (2, p. 94). Frogs are capable of revival after exposure to $-25^{\circ}\text{C}.$ for an hour, but when the temperature is taken by a thermocouple placed in the stomach, they die when it is kept at $-1.5^{\circ}\text{C}.$ for 2 hours, or at $-2^{\circ}\text{C}.$ for $1\frac{1}{2}$ hour (18). Frogs placed in carbon-dioxide snow for 8 minutes become hard and rigid, but recover movement and sensation on being warmed (2, pp. 84, 85). Fish frozen to stiffness but still flexible recover, but they die when frozen to fragility (19). Goldfish do not recover after immersion in liquid air for 15 seconds (20).

METHODS AND RESULTS

The following study of the resistance of nerve to ultra-freezing was made on frog sciatic-gastrocnemius preparations which had been dissected on the previous afternoon and preserved in dextrose-Ringer's solution in a refrigerator near $0^{\circ}\text{C}.$ (1). The excitability of the nerve and muscle was checked by a Harvard inductorium activated by a dry cell of about $1\frac{1}{2}$ volts, with the secondary coil at 12, 6 and 0 cm. The nerve was frozen by tying a thread to the Achilles tendon and dipping the whole length of the nerve for a specified time into a beaker of isopentane immersed in liquid nitrogen which produced a temperature of about $-160^{\circ}\text{C}.$ or by placing it directly in liquid nitrogen ($-195^{\circ}\text{C}.$). The muscle was kept above the level of the freezing liquid. After a specified time, the preparation was thawed in cold dextrose-Ringer's solution. The excitability of the nerve and muscle was tested at various intervals. The responses are scored as: *vg*, very good, good response with secondary coil at 12 cm. *g*, good, good response requiring 6 cm.; *fg*, fairly good, good response requiring 0 cm.; *f*, fair, fair response at 0 cm.; *sl*, slight, slight response at 0 cm.; *O*, inexcitable at 0 cm.

Results of freezing frog sciatic nerve in ultra-cold isopentane. Immersion of the nerve for 10 seconds lowered the response from *vg* to *g*. Nerves immersed for 2

minutes are inexcitable immediately after thawing in cold Ringer's solution. If the freezing is not longer than 3 minutes, complete or almost complete recovery occurs within 8 minutes, and the response may remain fairly good for 3 days. The muscle then becomes inexcitable even to direct stimulation, which is much earlier than with preparations which have not been frozen. With immersion of 5 to 16 minutes there is considerable recovery in some cases, but in most others excitability is irreversibly abolished.

Non-immersed muscle was more or less injured by immersion of the nerve in ultra-cold isopentane even for three minutes, as manifested by early partial deterioration of the response to direct stimulation and by relatively early inexcitability. The inexcitable muscle was opaque and hard, as if in firm rigor. These changes were probably due to exposure of the muscle to the cold atmosphere above the isopentane.

Direct immersion of the muscle in ultra-cold isopentane for 10 seconds (rectus abdominis) or 5 minutes (gastrocnemius) destroyed its excitability completely and irreversibly. Muscle is therefore more vulnerable than the nerve. The texture of the muscle was also changed. It was rigid, contracted, hard and leathery. This rigor was not resolved by 4 days in cold Ringer's solution.

Nerve and muscle immersed in isopentane at room temperature for 15 minutes gave very good response, but when the immersion continued for 27 minutes, the response to both nerve and muscle stimulation was only fair, and after 40 minutes' immersion it was only slight. The muscle and possibly the nerve are therefore injured by isopentane at room temperature, but this becomes manifest only if the immersion is prolonged to more than 15 minutes. The injury by ultra-cold isopentane is therefore due to the freezing and not to the isopentane.

Direct immersion of frog sciatic nerve in liquid nitrogen for 1 minute had no effect on the excitability of the nerve or muscle, after thawing in Ringer, both giving τg response at 5 minutes and in a day. Both became inexcitable in 3 days. Immersion for 2 minutes abolished the response to nerve stimulation completely and irreversibly; the response to direct stimulation of the muscle remained good for 4 days, but was abolished on the 6th day. Freezing in liquid nitrogen therefore seems to be more promptly injurious to the excitability of the nerve than is freezing in isopentane.

Immersion of frog sciatic nerve in ethyl alcohol (95%) An answer to the question whether isopentane as such plays a material rôle in the injury of ultra-freezing was also sought by comparison with ethyl alcohol, an agent known to be directly injurious to nerve. The time for injury by this agent at room temperature was determined by stimulating the nerve of a preparation at successive intervals after immersion, without rinsing in Ringer. The response declined from τg before immersion, to g after immersion of 2 to 4 minutes, fg after 6, 8 and 10 minutes, and to inexcitability in 15 minutes. This inexcitability was irreversible by transfer to cold Ringer for one hour to 3 days. The room temperature injury with ethyl alcohol is definitely greater than with isopentane; for isopentane still gave τg response at 6 minutes, when the alcohol had declined to fg ; and the alcohol nerve was inexcitable at 15 minutes, while the isopentane nerve gave an f response at 27, and a slight response at 40 minutes. The early injury by ethyl alcohol as such restricts its use

for ultra-freezing to short periods which, according to the experience with isopentane, would only give some degree of reversible depression.

Such proved to be the case, with immersion of the nerve in alcohol cooled to about -100° C. by liquid nitrogen. The results of these brief immersions were of about the same order as those with ultra-cold isopentane, in harmony with the conclusion that the injury by ultra-freezing in isopentane is due to the cold and not to the substance as such.

Dehydration of the nerve with honey. Since injury by freezing is due in large part to mechanical trauma from ice crystals, partial dehydration increases the resistance of some isolated tissues (2, p. 60). This was tried on frog sciatic nerve by laying it in honey. The high content of invert sugar (65 to nearly 80%; U. S. Dispensatory) promptly withdraws water and presumably some electrolytes very effectively, without producing direct toxic actions. It was necessary first to determine the effects at room temperature. The nerves of the sciatic-gastrocnemius preparations were laid in honey in a Syracuse watchglass, leaving the muscle outside. The preparations were kept in a moist chamber at room temperature and their excitability tested from time to time. When this had decreased materially, the preparation was transferred to Ringer's solution until its response was restored.

Almost immediately after the nerve is laid in the honey, the muscle begins to twitch for several minutes, then relaxes. The twitching may resume briefly when the nerve is transferred to the Ringer's solution, after 15 minutes in the honey. The physical characters of the nerve undergo striking changes in the honey, becoming translucent, stiff, horny and brittle. These alterations set in within 7 minutes when the response is still fairly good. On transferring the nerve back to Ringer's solution it resumes its normal appearance, its softness and its pliability quite promptly. The restoration is perceptible in 4 minutes and complete in 15 minutes.

The response to nerve stimulation decreases rather slowly during the immersion in honey, the rate differing considerably in different preparations, but all become inexcitable in 30 minutes to an hour. The decline may start within 5 minutes, or the response may be almost or quite unimpaired in 20 or 30 minutes and then disappear by 45 or 60 minutes. On transferring the nerve back to Ringer's solution the response begins to improve promptly. A preparation which had decreased to *fg* by 21 minutes in honey returned to *g* within one minute in Ringer. With two others, where it had declined to *fg* in honey for 45 and 60 minutes, transfer to Ringer caused little or no restoration of response in 15 minutes, but good response in 30 to 45 minutes.

Ultrafreezing of frog sciatic nerve in isopentane after dehydration in honey. When it was confirmed by the physical characters as well as by the decrease of excitability, that immersion of the nerve in honey produces prompt dehydration, a series of nerves that had been dehydrated in honey for 20 to 30 minutes, and which still gave *vg* or *g* response, were suspended in isopentane cooled by liquid nitrogen for 5, 10 and 15 minutes, then transferred to cold Ringer's solution and their excitability checked at 15 to 60 minutes. The results show that the dehydration did not increase the resistance of the nerve to injury by ultra-freezing. With 5-minute freezing, the responses after previous immersion in honey for 20 or 25 minutes are of the same

order as those without honey. With freezing of 10 and 15 minutes, none of the honey preparations was excitable, while in each case one of the two non-honey preparations gave good response. The number of experiments is not sufficient to prove that the dehydration and freeze injuries are additive.

SUMMARY

Ultra-freezing of frog nerve and muscle at about -160° C. by immersion in isopentane cooled by liquid nitrogen, or at -195° C. by direct immersion in liquid nitrogen, abolishes their excitability promptly, completely, and generally irreversibly; recovery depending on the time of immersion, presumably according to whether the cold has penetrated to all the fibers.

Frog sciatic nerve frozen for 2 minutes in the isopentane remains inexcitable for several minutes of thawing in cooled Ringer's solution, but recovers fully within 15 minutes. When frozen for 3 minutes, it also recovers largely. When frozen for 5 minutes, recovery in Ringer's solution is generally less complete, and of relatively short duration; the response decreasing again within an hour, sometimes to complete extinction. Freezing for 10 to 16 minutes generally abolishes the response permanently, but one preparation recovered almost completely and still gave as good response after an hour in Ringer's solution.

Skeletal muscle is definitely more vulnerable to ultrafreezing than is the nerve trunk. Immersion of the frog gastrocnemius in the cooled isopentane for 5 minutes destroys its excitability completely and irreversibly, with parallel physical changes. The muscle, after thawing in Ringer's solution, is contracted, rigid, hard and leathery. The rigor is not resolved by 4 days in cold Ringer's solution.

The changes in the nerve and muscle are not due to the isopentane as such, for immersion at room temperature for 15 minutes does not impair their response. However, with 27 and especially with 40 minutes of immersion their response is markedly decreased.

Freezing by immersion in liquid nitrogen produces the same changes in the nerve as does ultra-cold isopentane, in a somewhat shorter time. Ethyl alcohol (95%) at room temperature materially depresses the response within 6 to 10 minutes of immersion of the nerve. Immersion of 15 minutes abolishes the response completely and irreversibly. It is therefore much more injurious than isopentane. Freezing for short periods in alcohol cooled to about -100° C. gives about the same results as ultra-cold isopentane.

Dehydration of frog sciatic nerve by immersion in honey alters its physical characters, so that it shrinks and becomes horny, translucent and brittle. These changes are reversed by returning it to Ringer's solution. Excitability is rather promptly depressed and abolished. Within an hour at most it is largely restored by transferring the nerve back to the Ringer's solution.

The honey-dehydrated nerve is not more resistant to the injury of freezing than is the normal nerve.

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STARLING'S LAW AND X-RAY DENSITY CHANGES OF HEART SHADOW¹

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THE factors which determine the stroke of the heart are the length of the muscle fibers (venous load), the arterial pressure and the composition of the interstitial fluid. The importance of the first two factors is implied in Starling's law which says that "the energy of contraction is a function of the length of muscle fibers." In the isolated heart, the composition of interstitial fluid remains reasonably constant so that the length of the muscle fibers and the arterial pressure are the determinants of stroke volume. Evidence both supporting and denying the importance of Starling's law for the intact animal has been supplied from many laboratories (1-9). It is certain that the interstitial fluid in the heart of the intact animal is constantly undergoing changes not only because of alterations in size of coronary vessels and pressure within them but also as a result of variations in nervous impulses and the composition of the blood. Is the stroke in the intact animal determined largely by the composition of interstitial fluid or by the venous pressure which stretches the muscle fibers? With a new instrument, the electrokymograph, we hope to add to our understanding of these factors.

For this research the anaesthetized dog was chosen because humoral factors are less variable than in normal animals where nervous influences are constantly causing changes in blood flow and composition. To prove Starling's law for the intact animal, one must be able to show that an increase in work of the heart which is brought about by an increased stroke and/or increased arterial pressure is accompanied by a dilation of the heart. If one could place the heart in a volume recorder this would be easy. But one must rely on indirect methods. Perhaps the best one is x-ray shadows of the heart (4). However, the changes here are so small and occur so rapidly that they are difficult to measure accurately. Furthermore one records the size in only one plane at a time. With the electrokymograph (see below) one can amplify the border movements of the heart and can even record changes in x-ray density at the middle of the heart's shadow. When one records from a large part of the ventricle, the curve should represent a reasonable average for border movements during the cardiac cycle. Such records taken with measurements of the stroke of the heart should give a means of telling whether or not the size of the heart varies with the stroke. The principle involved is illustrated by the following example. A heart having a volume of 700 cc. (blood and muscle of left and right side) and ejecting 200 cc. will show a smaller border movement than one having a volume of 500 cc. and also ejecting 200 cc. Thus if the heart dilates with increasing strokes, the border movements per unit of

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output should become smaller. If the heart does not dilate, then the border movements should remain approximately the same per unit output. If Starling's law holds, the former effect should be observed. Admittedly the mathematics is not as simple as is implied here. Evidence from this method is to a considerable extent empirical.

METHOD

To measure the output of the heart, we have used two methods—the ballistocardiograph and that of pulse pressure. The ballistocardiographic table was of the low frequency type similar to the one described by Nickerson and Curtis (14). For studies on the dog, there is no formula for converting the ballistic forces into stroke volume. We have, however, performed enough experiments measuring heart output by the direct Fick method, while recording almost simultaneously the ballistic forces, to feel confident that a formula can be devised. In our calculations, we have been interested only in changes in heart output and therefore have used no constants to give our records meaning in terms of actual output.

The other method used for determining changes in stroke volume was described by Hamilton and Remington (10). It is based on a study of contours of arterial pressure tracings. Pulse pressures were recorded with a strain gauge (Stratham laboratories, Model P 23-150-250 Ser. 60). The pressure was transmitted to the gauge through polyvinyl tubing which had been baked to harden it. This tubing was 0.5 mm. in diameter and 25 cm. in length.² One end was pushed through the carotid artery so that it extended into the aorta. The other end was connected with the gauge. A string galvanometer recorded pressure.

The movements of the heart borders were recorded with the electrokymograph (11). This instrument consists of a fluorescent screen placed over the light-sensitive portion of a multiplier phototube (RCA 931-A). When x-rays act upon this screen, fluorescent light is emitted. This light in turn is converted into an electrical current. If the x-rays vary in intensity, the intensity of fluorescent light also varies. The changes in current from the phototube are recorded with a string galvanometer. No light from the room can reach the photosensitive surface of 931-A tube because the only opening in the tube housing is covered with black paper. By fluoroscopic guidance the area of the pick-up which responds to x-rays is placed so that it is covered completely or partially by the heart shadow. In either case, the records obtained look much like those recorded by a cardiometer. When the area studied is within the heart shadow, the record is one of changing x-ray opacity of the heart and has been called a density curve or a densogram. The densograms have been used for this study. The area of the heart shadow from which the records were made was 2 cm. in diameter.

In order to vary the stroke of the heart, the right vagus nerve was stimulated, thus slowing the rate of the heart and allowing more time for filling. Simultaneous records of the ballistocardiogram, the pulse pressure and the electrokymograms were made. The results obtained from at least five beats of similar size have been averaged in calculating the points on the graphs.

² Suggested by L. H. Peterson, University of Pennsylvania.

The major part of the work of the heart may be calculated by multiplying the stroke by the mean pressure occurring during ejection. A small additional figure should be added to account for the velocity imparted to the blood. This velocity factor is small and difficult to calculate so it has been omitted. Also we have made no estimate of the work of the right heart. The work of the left heart was assumed to parallel that of the right. The work then was calculated by multiplying the stroke as determined by ballistocardiograms or pulse pressures by the mean systolic pressures.

Epinephrine increases the force of cardiac contraction (1, 12). Therefore when this drug is given, the heart should require less filling to do its usual work or should be able to do more work with the same filling. The effect of vagal stimulation after giving epinephrine intravenously has been investigated.

RESULTS

A record showing the effect of vagal stimulation on ballistocardiograms, blood pressure and EKY density is shown in figure 1. It will be noted that following vagal stimulation, all of the records show increased amplitudes.

The significance of these results is shown by plotting the work accomplished against the density changes. In figure 2 (the lower curves) this has been done. The work of the heart per beat is shown on the abscissas and the density change per unit output on the ordinates. Slowing the heart by vagal stimulation increased the stroke and the work of the heart per beat. The observations recording the first beats after this stimulation appear in the right lower portions of the graph. During the period of increasing rate, the work per stroke decreases and these results appear higher and to the left.

If the heart did not dilate with increasing work, then the border movements or density changes would be proportional to the stroke, and the line connecting the points would be horizontal. On the other hand the more this line approaches the vertical the greater the dilation produced by additional work. These records indicate that the heart does dilate with increasing work as is to be expected from Starling's law.

The observations made immediately after intravenous epinephrine appear on the graphs above the control observations. They indicate greater x-ray density changes for a given stroke which is evidence that the heart has less residual blood in it when under the influence of epinephrine. Or we may say that for a given length of muscle fibers, it contracts more vigorously under the influence of epinephrine. During the recovery period it will be noted that the curve drops back to the control levels once more.

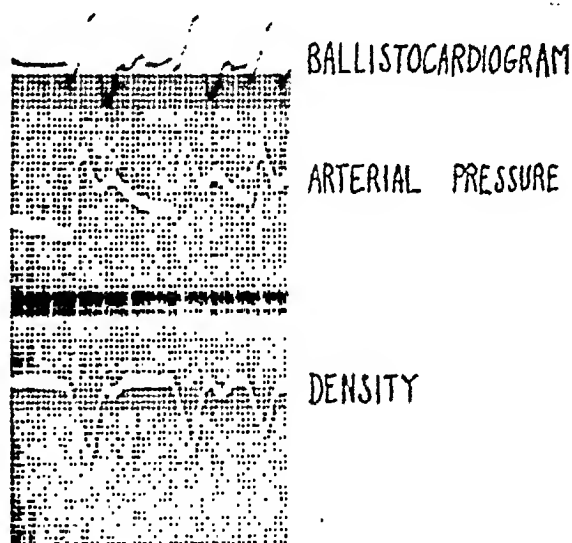


FIG. 1

DISCUSSION

The density changes recorded are not parallel with stroke-volume. In figure 3 the stroke volumes were plotted against the density changes per unit output. It is

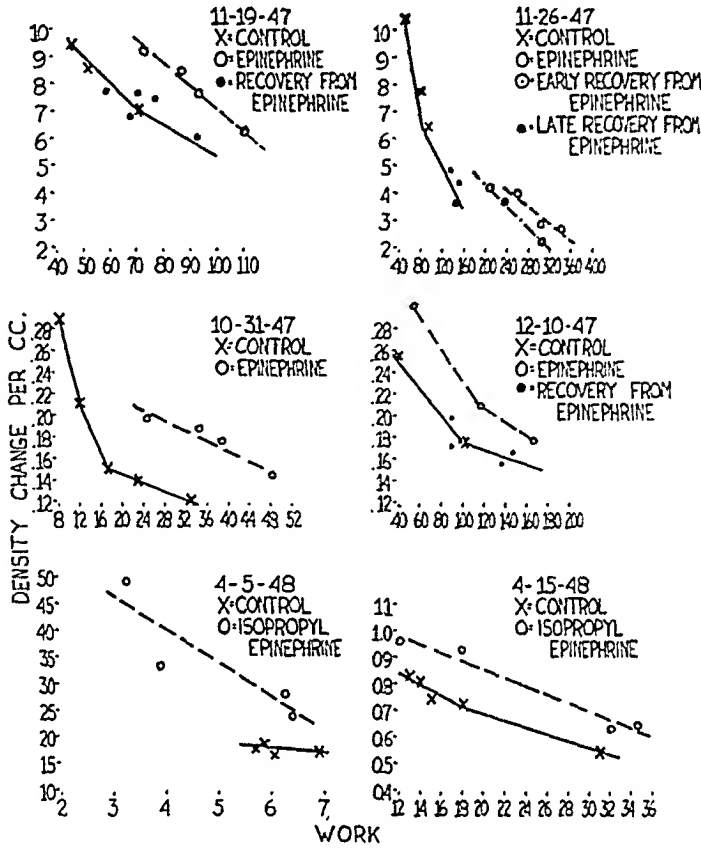


FIG. 2

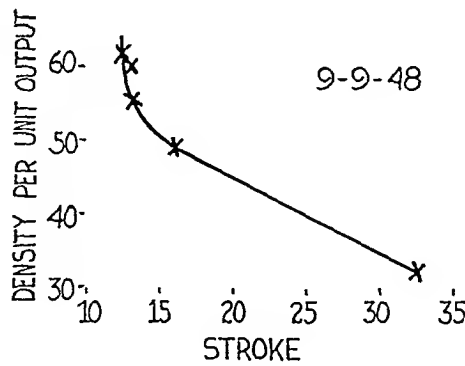


FIG. 3

seen at once that the points do not give a horizontal line but rather a falling line with increasing stroke. It then becomes important to determine whether the density records show changes proportional to the border movements of the heart. Roentgenkymography seemed to offer our most likely answer to this question. Such a record from the dog's heart after vagal stimulation is shown in figure 4. It will be seen that the border movements are largest immediately after vagal stimulation and become

smaller thereafter. The records are quite similar to those obtained with the electrokymograph (fig. 1). One would like to have simultaneous roentgenkymograms and electrokymograms but with our equipment we had to be satisfied with successive records. Our comparison has been made between the first and second beats after vagal stimulation. In 4 dogs, the roentgenkymograph records show that the amplitude of the inward movements of the heart shadow was smaller for the second beat



FIG. 4 MOVEMENTS OF VENTRAL BORDER shown above and dorsal border below.
Read from left to right.

after vagal stimulation than for the first. The second beats were on the average 82 per cent as large as the first beats. In these same dogs densograms of second beats were 70 per cent of the size of first beats. A mathematical analysis of the relation between electrokymograms and roentgenkymograms indicates that the relation found is approximately the one to be expected. It would appear then that the electrokymograph gives a satisfactory measure of border movements under the conditions of this experiment.

After exercise in man, the stroke and density changes per cc. of output diminish during recovery. This is exactly what is to be expected from Starling's law. If the

sympathetics were important in determining the large stroke after exercise (1, 13), it would seem at least possible that the heart would show more rather than less dilation as recovery progressed. In 28 subjects carefully studied, this has never been observed.

SUMMARY

Utilizing electrokymography, a method has been described which, it is believed, will record rapid changes in the size of the heart within the intact chest. This method has been used to show that in the intact animal the heart follows Starling's law during the period after vagal stimulation. Under epinephrine, the heart responds to a given load of work with less dilation than under control conditions.

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EFFECT OF BLOOD SUGAR LEVELS AND INSULIN LACK ON GLUCONEOGENESIS BY THE KIDNEY OF THE DOG

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BY A variety of methods, the kidney has been shown to be a source of blood sugar. Bergman and Drury (1) noted that greater amounts of glucose, given by a constant intravenous injection, were required to maintain a constant normal blood sugar level in liverless nephrectomized rabbits than to maintain a constant level in rabbits that were merely hepatectomized. The difference between the two types of animals, in the amount of injected glucose required, has since been considered to represent the glucose output of the kidney. By analysis of simultaneous arterial and renal venous blood sugar concentrations in rats and dogs, Reinecke directly demonstrated the gluconeogenic function of the kidney (2-4). Significantly higher glucose concentrations were found in renal venous blood than in the arterial samples. Others have noted glucose production by renal tissue *in vitro* (5). The factors involved in regulating glucose production by the kidney have not as yet been demonstrated, except for the work of Russell (5), who found a decrease in the ability of kidney from adrenalectomized rats to form glucose. The results reported below are studies of the regulation of renal gluconeogenesis.

METHODS

The renal contribution to blood sugar was evaluated by comparing the rate of fall of blood sugar in liverless animals in the presence and absence of the kidneys. This procedure of following the rate of decline in blood sugar was performed in normal and previously depancreatized dogs.

The effect of induced hyperglycemia was studied by injecting a single intravenous dose of glucose into hepatectomized normal animals immediately after operation. These animals also were divided into two groups, those with and those without kidneys. Diabetic dogs had been depancreatized three days before hepatectomy. The techniques for evisceration and true blood sugar determination have been previously described (6-8).

Starting with an immediate post-evisceration sample, blood for the determination of glucose was collected at 30-minute intervals until death. The results are plotted as percentage of the blood sugar value found immediately after evisceration or 10 minutes after the glucose injection.

RESULTS

The rate of fall of blood sugar in liverless animals without kidneys was approximately twice as rapid as in liverless animals with kidneys. This finding was observed in both normal and diabetic dogs (fig. 1, table 1). The survival time of both nephrectomized and non-nephrectomized animals paralleled the decline in blood sugar. Animals with intact kidneys lived approximately twice as long as nephrectomized

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animals. An initial hyperglycemia, produced by a single injection of glucose into normal hepatectomized animals without kidneys, did not influence either the rate of fall of blood sugar or the survival time. The least rapid rate of fall in blood sugar, when all groups studied were compared, was found to be in the hepatectomized normal dogs with functioning kidneys given an injection of glucose (fig. 2).

TABLE I. RATE OF FALL OF THE BLOOD SUGAR, AS PERCENTAGE OF THE IMMEDIATE POST-OPERATIVE SPECIMEN, IN EVISCERATED HEPATECTOMIZED DOGS (E-H) AND IN EVISCERATED HEPATECTOMIZED NEPHRECTOMIZED DOGS (E-H-N)

TIME ²	TYPE OF DOG					
	NORMAL				DIABETIC	
	Conditions					
	Not given glucose		Given i.v. glucose ¹		Not given glucose	
	E-H	E-H-N	E-H	E-H-N	E-H	E-H-N
<i>min.</i>						
0	100 ₁	100 ₂	100 ₃	100 ₄	100 ₅	100 ₆
30	76(±2)	60(±9)	78(±4)	66(±3)	85(±2)	74(±3)
60	61(±2)	45(±5)	65(±5)	47(±5)	72(±5)	52(±5)
90	47(±3)	32(±7)	51(±7)	33(±7)	57(±3)	37(±5)
120	44(±5)	16(±4)	44(±6)	19(±5)	43(±3)	23(±4)
150	26(±3)	7(±6)	34(±6)	3(±2)	34(±4)	22(±3)
180	22(±4)		27(±7)		26(±4)	11(±5)
210	19(±6)		23(±8)		20(±4)	
240	14(±3)		20(±8)		13(±7)	
270	12		14(±7)		10(±4)	
300	12		10(±6)			
330			6(±4)			
360			6(±4)			
390			5			
420			4			

¹ $\frac{1}{2}$ gm. of glucose per kilo I. V. immediately after operative procedure.

² Time after the first post-operative blood sugar was taken. Actual average blood sugar concentration = ₁ = 97 mg%; ₂ = 78 mg%; ₃ = 249 mg%; ₄ = 232 mg%; ₅ = 304 mg%; ₆ = 272 mg%.

The figures given in each column are the mean values; those in parentheses are the standard deviations.

DISCUSSION

The kidney and the liver, both sources of blood sugar, might or might not respond identically to disturbed homeostasis. Both organs could store glucose under the same conditions or they could act so as to complement each other, the one storing glucose while the other was releasing it. Hyperglycemia has been shown by Soskin *et al.* (9) to result in the hepatic storage of glucose; hypoglycemia had the opposite effect.

Endocrine control of hepatic glucose production is not well understood. The results obtained by Crandall (10) in normal and depancreatized dogs yielded no evidence for over-production of glucose by the livers of diabetic dogs. In human

beings, however, the published results indicate the liver to be the site of the overproduction of glucose seen in the diabetic state (11).

Our results indicate that blood sugar levels have little effect upon renal gluconeogenesis. The normal kidney releases glucose to the blood at all blood sugar levels studied (7-300 mg. %). Within 30 minutes after evisceration, the blood sugar of the liverless nephrectomized animal had already fallen more rapidly than that of its mate with intact kidneys. This result was observed both in normal dogs at normal blood sugar levels (60-90 mg. %) and in normal dogs made hyperglycemic (blood sugar = 200-300 mg. %) by one injection of glucose. Since peripheral utilization

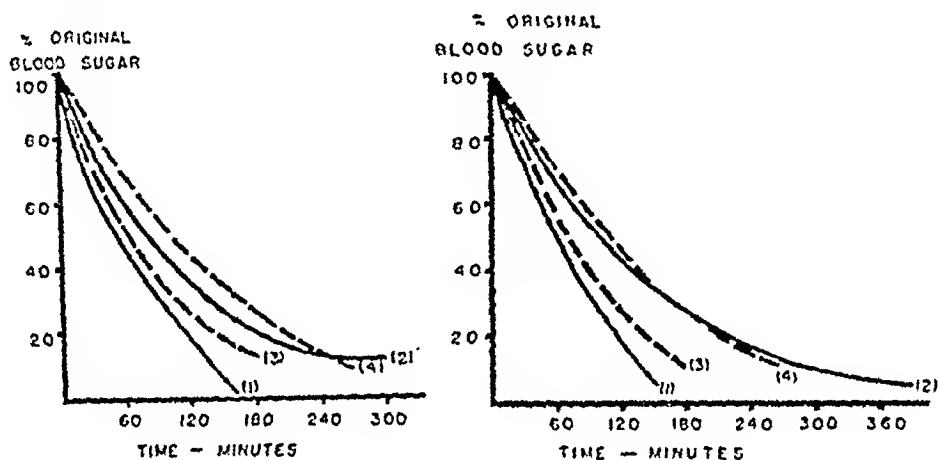


Fig. 1 (left). AVERAGE RATE OF FALL of blood sugar in normal and diabetic liverless dogs in the presence and absence of the kidney. (1), results of 7 normal hepatectomized nephrectomized dogs. (2), results of 7 normal hepatectomized dogs with intact kidneys. (3), results of 6 diabetic hepatectomized nephrectomized dogs. (4), results of 7 diabetic hepatectomized dogs with intact kidneys.

Fig. 2 (right). AVERAGE RATE OF FALL of blood sugar in normal liverless dogs, made hyperglycemic with one intravenous injection of glucose, and in diabetic liverless dogs, in the presence and absence of the kidney. (1), results of 4 normal hepatectomized nephrectomized dogs given one injection of glucose. (2), results of 4 normal hepatectomized dogs with intact kidneys given one injection of glucose. (3), results of 6 diabetic hepatectomized nephrectomized dogs (not given glucose). (4), results of 7 diabetic hepatectomized dogs with intact kidneys (not given glucose).

of glucose increases with higher blood sugar levels (12), it is even possible that the kidney releases more glucose at higher blood sugar levels than at low ones.

Our results do not indicate control of renal gluconeogenesis by insulin. The rate of fall of blood sugar in the liverless diabetic dog is slightly less than in the normal hepatectomized animal. This difference is of the same order of magnitude as that observed when the curve of decline in the blood sugar of the diabetic nephrectomized dog is compared to that of the normal nephrectomized animal. If the kidney of the depancreatized animal were the site of increased gluconeogenesis, a much slower fall in blood sugar should have occurred after hepatectomy. In addition, the eviscerated diabetic dog at its normal blood sugar level (250-350 mg. %) utilizes approximately the same amount of glucose as does the normal animal at its normal blood sugar level (60-90 mg. %) (12). If the kidney of the normal and diabetic animal were to produce the same amount of glucose, the rate of decline in the blood sugars should parallel each other. The results given in figure 1 show this to be the

case and may be interpreted as indicating similar rates of glucose production by the kidney of the normal and depancreatized dog.

SUMMARY

The rate of decline of the blood sugar in hepatectomized dogs is more rapid in nephrectomized animals than in those with intact kidneys. This is true both in the normal and diabetic dog. Renal gluconeogenesis in normal animals occurred irrespective of the blood sugar levels. The same amount of sugar appears to be contributed to the blood by the kidneys of normal and of untreated diabetic dogs.

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PANCREATIC DIABETES IN THE CALF

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A SURVEY of the literature on the effects of pancreatectomy reveals rather striking species variations which may be correlated with the natural dietary habits of the animals. Minkowski (1) and subsequent investigators found that dogs, cats and other carnivores develop a severe diabetes following removal of the pancreas. Under similar conditions, Carlson *et al.* (2) and Lukens (3) observed much less marked diabetic symptoms in the pig. Lukens (4) also studied a strictly herbivorous animal, the goat, and noted that the diabetic symptoms were not unlike those observed in the omnivorous pig. Greeley (5) obtained only a mild diabetic state in the depancreatized rabbit. These and numerous other papers suggest that such species differences may be correlated with the endocrine balance which physiologically regulates the underlying metabolic processes. To obtain additional information concerning species characteristics in intermediary metabolism and its endocrine control, the following experiments on the carbohydrate metabolism of normal and depancreatized calves were undertaken.

MATERIALS AND METHODS

Three young male calves, 2 to 3 weeks of age, were employed in these investigations. The diet consisted of whole milk throughout the experiments; this was supplemented with pancreatin (Merck) after pancreatectomy. After about a week of preliminary training in each case, control blood and urine values were obtained. Blood samples were taken from the jugular vein. By the use of elevated stanchions, continuous collections of urine could be made.

When the animals were 5 to 7 weeks of age, complete pancreatectomy was performed under nembutal anesthesia; 20 mg/kg. of body weight gave excellent results. Operative recoveries were uneventful in calves 2 and 3; calf 1 developed congestion of the lungs and died on the 2nd postoperative day.

The Somogyi-Shaffer-Hartmann method (6) was used for the quantitative determination of blood and urine glucose. Urinary nitrogen values were obtained by the macro-Kjeldahl procedure (7). Urine ketone bodies were determined, as acetone, by the method of Ravin (8). Liver and muscle glycogen percentages were obtained by the Good-Kramer-Somogyi method (9).

RESULTS AND DISCUSSION

Blood Glucose. The average blood glucose level of the normal calves was found to be 81 mg/100 ml. During the first 42 hours of fasting this dropped to an average of 49 mg/100 ml. With continued fasting, however, a compensatory¹ rise in the glycemic level occurred at about the 67th hour, and was followed by a secondary fall in 2 out of 3 cases (table 1). It was found that the animals could be fasted for long periods, 4 to 6 days, without the hypoglycemia becoming extreme.

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Following pancreatectomy, on the other hand, the glycemia was roughly proportional to the food intake (figure 1). The hypoglycemia which developed when these animals were fasted was more extreme and occurred more rapidly than that seen in

TABLE 1. EFFECT OF FASTING ON NORMAL CALVES

HOUR	BLOOD GLUCOSE, MG/100 ML.			URINE NITROGEN, GM/KG/DAY		
	Calf 1	2	3	1	2	3
0	82	86	76	0.13	0.23	
19			77			0.28
43	56	48	42	0.20	0.27	0.60
67	66	61	47	0.26	0.32	0.60
91	70	53	52	0.26	0.34	0.83
115			45			0.37
139			41			0.57

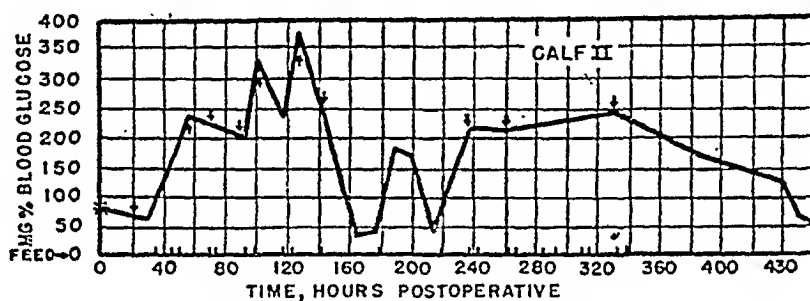


TABLE 2. EFFECT OF FASTING ON DEPANCREATIZED CALVES

HOUR	BLOOD GLUCOSE, MG/100 ML.			URINE NITROGEN, GM/KG/DAY			URINE GLUCOSE, GM/KG/DAY		
	Calf 2	2	3	2	2	3	2	2	3
0	251	231	200	0.35			5.16		9.64
10	171	168							
19			148						0
24	33	49		0.31	0.23		0.70	0.30	
29			47						
37	36								
53			40 ¹						0
67			27						

¹ Fed 1 l. milk after this sample.

normal calves under similar conditions (table 2). On one occasion the fasting hypoglycemia in calf 3 was 27 mg/100 ml. before the fast was terminated by feeding. Fasts longer than 2 to 3 days were not attempted in the diabetic animals.

The average prefeeding morning-value of blood glucose in the depancreatized calves was 188 mg/100 ml. After maximal feeding the glycemia increased to 378 mg/100 ml. in one instance (fig. 1). An associated decrease in glucose tolerance in these diabetic calves was probably due to the same metabolic disturbance as the postprandial hyperglycemia.

The possible factor or factors involved in the production of diabetic hyperglycemia in the calf are obviously related to the diet. These may be one or more of the following: *a*) an increased rate of glucose absorption from the alimentary tract; *b*) an increased exogenous gluconeogenesis; or *c*) a subnormal utilization of glucose by oxidation and storage. The first possibility appears to be unlikely; the other two will be discussed below.

Urinary Glucose. Traces of glucose were excreted in the urine in both fed and fasted normal calves. In depancreatized diabetic calves, the glycosurias varied directly with the food intake and with the associated blood glucose levels (table 2). The maximal glycosuria observed in calf 2 was 5.16, and in calf 3 was 9.64 gm/kg/day. The fall of urine glucose in the fasting animals shows the failure of the diabetic calves to maintain a glycemic level above the renal threshold when endogenous gluconeogenesis is the sole source of blood glucose.

That gluconeogenesis was accelerated in the normal fasting calf, however, is indicated by the postprandial hyperglycemia and a glycosuria of 0.45 gm/kg/day after the first feeding at the end of a preoperative fast in calf 2.

Urinary Nitrogen. The usually accepted index to the magnitude of the gluconeogenic process from proteins is the quantity of nitrogen excreted in the urine. In the normal animal, the amount of nitrogen excreted was increased with the fasting time, as the blood glucose fell (table 1). When the fast was continued after the 90th hour in calf 3, the urine nitrogen values began to decline. Following pancreatectomy the average daily urinary nitrogen excretion was increased from 0.23 to 0.34 gm/kg. in calf 2, and from 0.28 to 0.32 gm/kg. in calf 3, increases of 48 and 15 per cent, respectively. The values are not sufficiently high quantitatively to indicate that more than a small fraction of the available glucose was being produced from protein. It seems probable, therefore, that the principal factor in producing the diabetic hyperglycemia in the calf is a diminished glucose utilization. An analysis of the data on glucose tolerance and postprandial hyperglycemia furnishes additional support for this conclusion.

It was also observed that instead of the usual increase in nitrogen excretion as in the fasted normal calf, there was, if anything, a small decrease in the fasted diabetic calf (table 2). It can be seen, therefore, that the increase in endogenous glucose production from protein in the diabetic animals was slight in comparison with the normal calves. A relatively low level of gluconeogenesis under these conditions may account for the hypoglycemia which develops during fasting. These results, which are in marked contrast to those found in carnivores, would seem to indicate that calves have a poor mechanism for mobilizing body proteins and fats for gluconeogenesis in comparison to that of the former group of animals.

Ketonuria. Although ketonuria was not observed in normal calves before pancreatectomy, slight amounts of ketone bodies were found in the urine of the depancreatized animals. The average quantity excreted by calf 2 was 0.97 (range 0.68-1.8) and by calf 3 was 0.54 (range 0.43-0.70) gm/kg/day. Fasting caused a complete disappearance of the ketonuria in calf 2, and a decrease to 0.32 gm/kg/day in calf 3. The low-grade ketosis accounts, in part, for the mildness of the diabetic syndrome in this species.

The above facts concerning ketonuria in diabetic calves may indicate: *a*) an unusually high level of ketone body utilization; *b*) a relatively low level of fat metabolism; or *c*) a source of ketogenic substances which is primarily dietary. Since it has been shown that the utilization of ketone bodies in the peripheral tissues is not affected by pancreatectomy in the dog (11, 12), the first factor may be considered improbable. Because the ketonuria is decreased or abolished by fasting, it seems

likely that the last factor is of primary significance. The levels of mobilization of body fat and of endogenous fat metabolism were apparently insufficient to produce a ketonuria. The observation that the livers of these diabetic animals were not heavily infiltrated with fat adds evidence that the level of fat metabolism was not greatly increased.

Glucose Tolerance. Because glucose is readily fermented in the alimentary tract of adult ruminants, oral glucose tolerance tests would be of little value in the study of carbohydrate metabolism in these animals. Since there appears to be no reliable information in the literature on when the rumen becomes functional in the young calf, intravenous glucose tolerance tests were employed; 1 gm. of glucose per kg. of body weight was injected intravenously and the subsequent blood glucose curve plotted by taking successive blood samples. Representative curves are shown in figure 2. Thirty minutes after the glucose injection in normal animals, the blood glucose level averaged 150 mg/100 ml. higher than the pre-

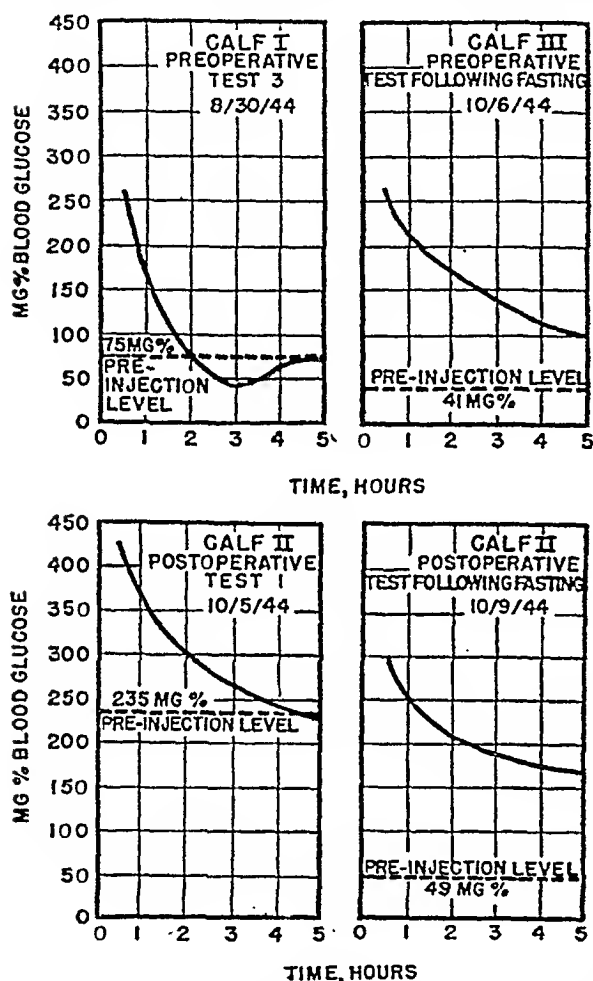


Fig. 2. GLUCOSE TOLERANCE curves following intravenous injection 1 gm. of glucose per kg. body weight.

injection level. After this, the curves fell with fair rapidity, and had similar slopes for all normal animals. The rate of utilization was apparently proportional to the concentration of blood glucose. In 1 to 2.6 hours the glycemic level had returned to normal. The curves then fell below the preinjection level where they remained for about 2 hours.

Glucose tolerance curves for unoperated calves following 5-day fasts showed a decrease in the rate at which the blood glucose returned to normal. The blood glucose failed to drop to the preinjection level during the usual period of sampling.

In the non-fasting tolerance studies following pancreatectomy, the glycemic level rose an average of 201 mg/100 ml. on the 30-minute sample. The curves were greatly prolonged and the blood glucose failed to drop to the preinjection level until after the 4th postinjection hour. The curve for the depancreatized animal following fasting was even more prolonged and failed to drop to the preinjection level during the period of sampling.

This prolonged curve in the depancreatized calf shows a marked decrease in its tolerance for glucose. Apparently this is due, in part, to the failure of the liver to maintain homeostasis and, in part, to the inability of the animal to oxidize and to store the excess glucose adequately (12).

The glucose excreted in the urine during these tests also serves as an indication of the tolerance for glucose. After subtracting the value of glucose excreted from that injected, it was found that an average of 90 per cent of the injected glucose was utilized in the normal calf. It is unfortunate that digestive disturbances caused fecal contamination of the urine of the depancreatized animal and thus prevented a similar analysis. On one occasion diabetic calf 2 excreted an equivalent of 74 per cent of the injected glucose on the day of a non-fasting glucose tolerance test.

Postmortem Observations. Prolonged survival studies were not carried out in these animals. During the course of the experiments they increased in size but lost weight after pancreatectomy. In large part this probably was due to digestive disturbances which resulted from the absence of pancreatic juice. In other respects, however, they were in fairly good condition and could, no doubt, have been maintained for a much longer time. Neither animal showed gross fatty changes in the liver. Autopsy revealed that pancreatectomy had been complete in each case.

Quantitative analysis of liver and muscle tissue gave glycogen values for calves 2 and 3 of 0.52 and 0.78, respectively, for muscle, and 2.28 and 2.55 per cent, respectively, for liver. These are within the normal mammalian range although those for liver are in the lower part of this range.

SUMMARY

The depancreatized calf exhibited only mild diabetic symptoms. Removal of the pancreas resulted in a fluctuation of the glycemic level above and below the average normal value of 81 mg/100 ml. in direct proportion to the food intake. In the fasting diabetic animal, the blood glucose dropped to more extreme hypoglycemic levels, and more rapidly than in the fasting normal calf. The glycosuria of the depancreatized calf also varied directly with food intake and could be abolished by fasting. Urinary nitrogen was not excessive in the diabetic and failed to increase during fasting as it had in the normal calf. Ketonuria was negligible under all conditions.

Glucose tolerance was lowered both by fasting and by pancreatectomy. Although the normal calf excreted very little of the glucose administered, non-fasting diabetic calf 2 lost 74 per cent in this manner. The terminal liver and muscle glycogen values were in the lower limits of the normal range. The livers were normal in color; no gross deposition of fat was visible.

These results indicate relatively low levels of endogenous mobilization and metabolism of protein and fat in the depancreatized calf.

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EFFECTS OF ALLOXAN ADMINISTRATION IN THE CALF

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THE observation of Cook, Dye and McCandless (1) that the blood glucose level of the depancreatized calf varies with the food intake, dropping to hypoglycemic levels when the calf is fasted, created further interest in the role of insulin and the endocrine balance on carbohydrate metabolism in the young ruminant. To investigate the influence of a pure hypoinsulinism upon the intermediary metabolism of the calf, alloxan monohydrate was employed as a diabetogenic agent in the experiments described below. Alloxan produces a selective necrosis of the beta or insulin-producing cells of the pancreatic islets with little or no degeneration of the other cellular components of the islets or of the acini (2).

METHODS

Four Guernsey bull calves, 2 to 3 weeks of age, were used in these experiments. The feeding routine, general experimental procedures and methods were similar to those described by Cook *et al.* (1). After control studies had been carried out, alloxan monohydrate (Eastman) was injected intravenously as a 5-per cent solution in sterile distilled water; the injection time was less than 5 minutes.

The blood glucose fluctuations which occur after alloxan administration were followed for 24 hours or longer. *Calf 1* received 65, 100, 150, and 175 mg. of alloxan/kg., at 4-, 6-, and 22-day intervals; *calf 2*, a single dose of 150 mg./kg.; *calf 3*, 100, 100, 100, 125, 125, and 150 mg./kg. at 2-day intervals; *calf 4*, 100, 125, 125, and 125 mg./kg. at 2-day intervals. In the attempt to produce a diuresis and thus to prevent alloxan-inflicted renal damage, which had proved fatal to *calf 2*, the last two animals were given 6 liters of diluted milk (1:2) 15 minutes before alloxan administration. Studies of blood and urine chemistry were carried out between alloxan injections to determine the efficacy of the drug as a diabetogenic agent in this species.

Pieces of pancreas, liver and kidney, removed immediately after death from *calves 2, 3, and 4*, were fixed in Bouin's and in Zenker's acetic solutions. Sections were stained with the routine hematoxylin and eosin technique. Through the kindness of Dr. Gomori (3), pancreatic sections were also prepared with the chrome-hematoxylin and phloxin stain.

RESULTS

Initial Glycemic Fluctuations after Alloxan Administration. With the exception of the guinea-pig, a rather typical triphasic glycemic fluctuation occurs within the first 24 hours after alloxan administration in all species reported to date. An initial hyperglycemic phase usually develops 1 to 2 hours postinjection and is followed by a secondary hypoglycemia. The third phase, diabetic hyperglycemia, is usually present 24 to 48 hours after a diabetogenic dose of alloxan has been given. In the guinea-pig this last phase is extremely slow to develop and is transitory (4).

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In the calf, however, the initial hyperglycemia was not observed after any of the 15 alloxan injections given to 4 animals (table 1). Although occasionally the blood

TABLE 1. INITIAL GLYCEMIC FLUCTUATIONS AFTER ALLOXAN ADMINISTRATION

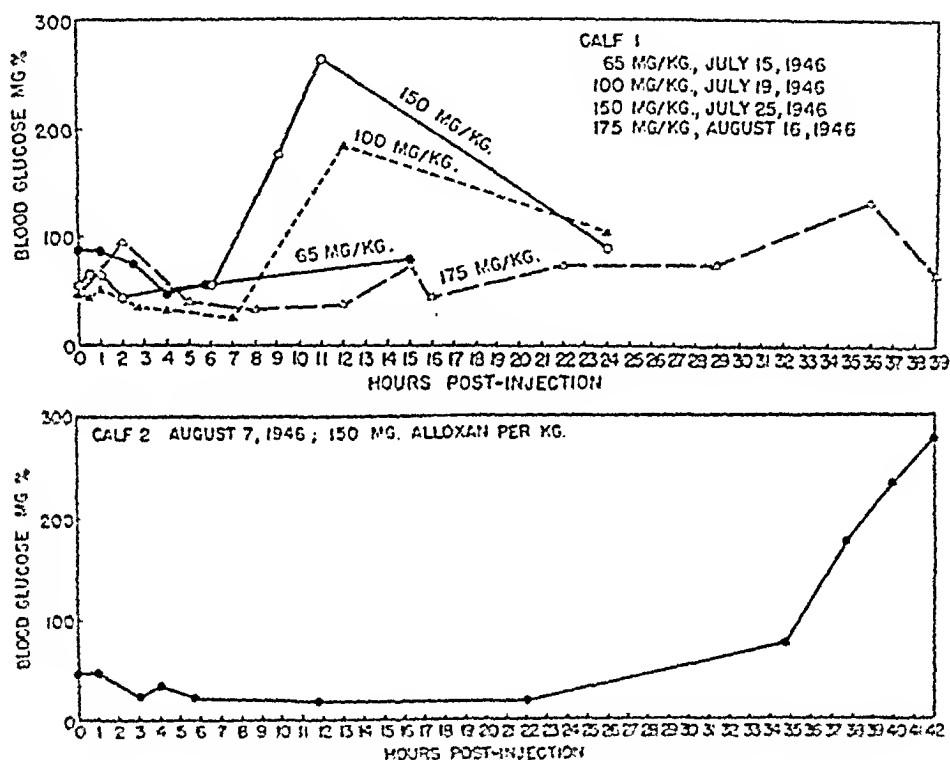
CALF.....	1				2	3						4			
Alloxan dose, mg/kg.....	65	100	150	175	150	100	100	100	125	125	150	100	125	125	125
Interval between doses, days		4	6	22			2	2	2	2	2		2	2	2
Postinjec- tion, hr.															
0	89	47	56	47 ¹	47	84 ¹	96 ¹	84 ¹	98 ¹	82 ¹	82 ¹	88 ¹	70 ¹	75 ¹	94 ¹
0.5		44	65												
1	87	51	65		47	92						92			
2			42	96		114	138	94	107	134	114	77	102	103	99
2.5	75														
3		35			24	75									
4	47	33	52		33	79	112	110	88	101	99	60	82	112	105
5				40 ¹											
6	56 ¹		56		22	79	84	84		77	86	72	68	94	99
7		26 ¹	1		2										
8				33 ²		70	84		42	82	77	72	33	101	96
9			177												
10						65									
11	1		263				88	99	38	77	84	72	29	94	101
12		184		38 ²	18 ²	58			1			1	1		
14						68	84	1							
15	79			75	2				154	63 ¹	77 ¹		103	88 ¹	96 ¹
16	1			45	2	72 ¹									
22				75	19 ²										
24	96 ¹	105	91			84 ¹	84 ¹	82 ¹	88 ¹	79 ¹	91 ¹	70 ¹	93 ¹	99 ¹	104 ¹
25					2										
26						116	116			105	127			182	173
29		1	1	75 ¹											
32		184	103												
35					75										
36				132											
38					147										
39				63											
40	98 ¹				233										
42	110				275										
44				70											
48		112	75			96	84	98	82	82		70	75	94	

¹ Fed. ² Intravenous glucose.

glucose level increased slightly following alloxanization, this must be attributed wholly to the postprandial hyperglycemia which would follow the preinjection feeding. The glycemic rise was less following feeding plus the alloxan injection than after feeding alone.

After each of the first 3 injections in *calf 1* and the single injection in *calf 2*, the blood-glucose level decreased steadily from the time alloxan was administered (figs. 1 and 2). In these cases, the animals had not been fed at the zero hour. A hypoglycemia below 50 mg/100 ml. was present in 2 to 4 hours. Hypoglycemia of this degree also developed in *calves 3* and *4* which were fed before the injections, but only after the 4th and 2nd injections, respectively.

The hypoglycemia was accompanied by severe convulsive symptoms in two instances, namely, after the administration of 175 and 150 mg/kg. in *calves 1* and *2*, respectively. These hypoglycemic convulsions, which are described in a preliminary paper (5), were relieved by the intravenous administration of glucose.



(Figs. 1 (upper) and 2 (lower). INITIAL RESPONSE TO ALLOXAN INJECTION.

Not only the initial hyperglycemia but also the tertiary phase, diabetic hyperglycemia, was missing in alloxanized *calves 1*, *3*, and *4*. The terminal rise in blood glucose which occurred in *calf 2* was the result of renal retention; this animal died 44 hours postinjection with complete coagulation necrosis of the renal convoluted tubules. In the other calves, the blood glucose returned to normal levels after the hypoglycemic phase.

Effects of Alloxan Administration. Although the doses of alloxan which were injected intravenously into *calves 3* and *4* were of the range which is diabetogenic for most species, histological examination of pancreatic tissue stained by the Gomori technique (3) showed no alterations typical of alloxan damage. Pancreatic islets present in the sections contained both alpha and beta cells. In *calf 4*, no pathological changes were observed. The pancreas of *calf 5*, however, gave evidence of functional stress, in that the beta cells of the islets were markedly degranulated.

No islets were observed in the pancreatic tissue of *calf 2*; however, it should be pointed out that this tissue may have been taken from the duodenal end of the pancreas rather than the splenic end, in which islet tissue is most abundant (6). Recognizing this possibility, sections were taken from the splenic end of the pancreas in the last two experiments. Unfortunately, no tissues were obtained from *calf 1*, as the animal died while on pasture approximately one year after the termination of the experiments; the carcass was not discovered until several days later.

It is apparent that the doses of alloxan used, i.e. from 65 to 175 mg/kg., were insufficient to produce complete alloxan diabetes, even though these doses were repeated frequently enough to have had a cumulative effect. Although beta cells were present in the pancreatic tissue of *calves 3* and *4*, postinjection observations indicated that the animals were abnormal in certain respects. Although postabsorptive hyperglycemia was absent in the 3 animals which survived alloxanization, postprandial

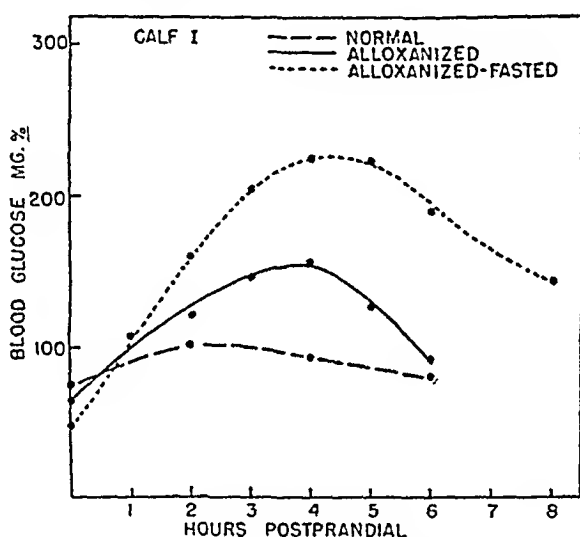


Fig. 3. POSTPRANDIAL BLOOD GLUCOSE.

hyperglycemia was observed on several occasions. In *calf 1* ninety-minute postprandial values of 184 and 135 mg/100 ml. were obtained after the dose of 100 mg. of alloxan/kg., and a value of 136 mg/100 ml. after the last dose, 175 mg/kg. The maximum blood glucose values of alloxanized *calves 3* and *4*, determined two hours after feeding, were 173 and 182 mg/100 ml., respectively. In normal calves which were given comparable quantities of milk, the maximal postprandial glycaemic levels occurred at the 2nd hour and did not exceed 125 mg/100 ml.

In the blood glucose curve determined in *calf 3* a week after the last administration of alloxan, the maximal glycaemic rise not only failed to occur until the 4th hour, but was much higher and of greater duration than the curves observed in normal calves (fig. 3). The same was true of the postprandial blood-glucose curve studied on the 1st day of feeding after a 4-day fast. It is therefore probable that the maximal postprandial hyperglycemia was missed in the earlier studies. Glycosuria was observed only in *calf 1*, and in this instance it was probably due to renal damage.

Another demonstration of the metabolic abnormality of alloxanized calves was obtained from intravenous glucose tolerance tests. *Calf 1* showed a temporary decrease in tolerance after the 3rd alloxan injection; a 4th dose of the drug produced a

permanently low glucose tolerance, still present 143 days later. Calves 3 and 4 also showed a low tolerance for injected glucose after the series of injections had been completed.

Aside from the postprandial hyperglycemia and the decreased glucose tolerance, however, no other manifestations of diabetes were observed. The urinary nitrogen excretion was not elevated significantly, nor was ketonuria present.

Although the doses of alloxan used were insufficient to destroy the insulin-producing cells of the pancreatic islets, severe renal damage was observed on histological examination subsequent to the death of the animals. Calf 2 died 44 hours after a single injection of 150 mg. of alloxan/kg. had been given, having exhibited toxic symptoms from the 38th hour. Anuria had been present from the time of the alloxan injection. The cause of death was found to be complete coagulation necrosis of the renal convoluted tubules. A similar, though less acute, disturbance developed in calf 1 after the administration of 175 mg. of alloxan/kg. Oliguria and nitrogen retention were severe, and hyperazotemia persisted for 7 months, when the blood NPN was 106 mg/100 ml. Although calves 3 and 4 were not azotemic, histological studies showed severe glomerular and tubular damage.

DISCUSSION

In view of the fact that normal beta cells persisted in the pancreatic islets of at least 2 of the 3 calves which survived alloxanization, and that the symptoms of both of these animals paralleled those of the other animal, calf 1, the conclusion seems justified that the calf is refractory to the diabetogenic action of alloxan in the doses given. The renal tissue of this species is apparently very sensitive to the destructive action of the drug, however. It is therefore doubtful that the calf can be used in further study of this form of experimental diabetes.

Nevertheless, it should be mentioned that the metabolic abnormalities which did develop in the calves are those of an incipient diabetes. It is interesting to note that Shipley and Rannefeld (7) observed a decreased glucose tolerance in rats treated with sub-threshold doses of alloxan. Saviano (8) reported an abnormal tolerance for injected glucose in a goat which was also refractory to the action of alloxan.

The most significant finding in this study, however, is the absence of the initial hyperglycemic phase and the rapid development of severe hypoglycemia after alloxanization. In this respect the normal calf bears a close resemblance to the hypophysectomized dog (9) and rat (10), and the adrenalectomized rabbit (11) and rat (10), none of which exhibit the initial hyperglycemia when alloxanized.

The observation of Cook *et al.* (1) that the glycemic level of the depancreatized calf is dependent upon the food intake lends further support to the theory that this species possesses a low degree of pituitary-adrenal function. The development of severe hypoglycemia in the fasting diabetic calf appears to be peculiar to the species, although the phenomenon is characteristic of the hypophysectomized diabetic (Houssay) dog.

SUMMARY

Four young bull calves failed to develop complete experimental diabetes after intravenous administration of single or repeated doses of alloxan ranging from 65 to

175 mg/kg., and total doses from 150 to 700 mg/kg. Mild postprandial hyperglycemia and decreased glucose tolerance were observed in the 3 calves which survived alloxanization. Severe renal damage was present in all of the animals. The normal calf, like the hypophysectomized or adrenalectomized dog and rat, fails to exhibit the initial rise of blood glucose after alloxanization.

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EFFECT OF EPINEPHRINE UPON THE TOLERANCE OF THE EVISCERATED RAT FOR GLUCOSE

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THESE studies show that epinephrine can affect the glucose tolerance of the eviscerated rat and that the nature of response is related to the experimental conditions. When glucose without insulin was administered to eviscerated rats the addition of epinephrine did not affect glucose tolerance during the first 2 hours but during 24 hours it caused glucose to disappear from the blood more rapidly. When glucose with insulin was administered to similar animals the addition of epinephrine caused a decrease in glucose tolerance within 2 hours which continued throughout 24 hours.

METHODS

Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At a weight of 185 to 205 gm., the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 (± 2) gm. they were anesthetized (intraperitoneal injection of 18 mg. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (1). Hemostasis was attained by applying a gelatin sponge (Gelfoam, Upjohn) saturated with a solution of thrombin to the stumps of the oesophagus, colon, ligated vessels and between the muscle and skin when the incisions were closed.

Solutions containing 0.9 per cent sodium chloride and varying concentration of glucose (C.P. Dextrose, Merck) with and without crystalline zinc insulin (Lilly) (4 U/24 hours/rat) and epinephrine hydrochloride (Upjohn) were infused into the saphenous vein of the right hind leg by means of a constant-injection machine which delivered fluid from each of 6 syringes at the rate of 20 cc. in 24 hours. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mg/100/h.). Insulin was given at the rate of 4 units per 24 hours. The infusions covered periods of 2 and 24 hours. Analyses of glucose were made on tail blood taken at the end of the infusion periods by the method of Miller and Van Slyke (2).

EXPERIMENTS AND RESULTS

Experiment 1 (fig. 1) covered a period of 2 hours. Twelve pairs of rats were represented in each group. Five groups of rats were given a glucose load of 16/100/h. without insulin. Epinephrine concentrations of 1, 10, 20, 40 and 50 parts per million were tested and caused no significant change in the level of blood glucose. Three

groups of rats were given a glucose load of 72/100/h. with insulin. Epinephrine concentrations of 10, 20 and 40 parts per million each caused a significant rise in the level of blood glucose as compared to that of the control animals.

Experiment 2 (fig. 2) covered a period of 24 hours. At glucose loads of 2, 4 and 6/100/h. without insulin all of the animals given epinephrine died of hypoglycemia. Three groups of rats were given glucose without insulin. *Group 1* received 8/100/h.

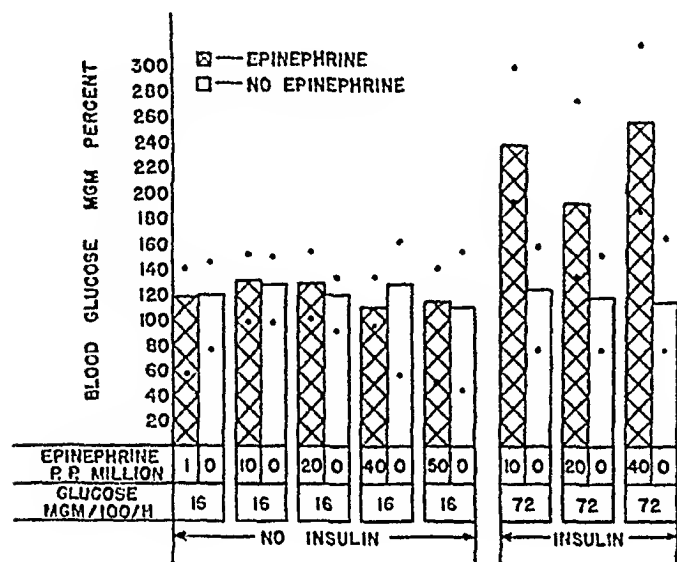


Fig. 1. Effect of epinephrine upon the level of blood glucose at the end of 2 hours of infusion. Averages and range. Twelve rats per group.

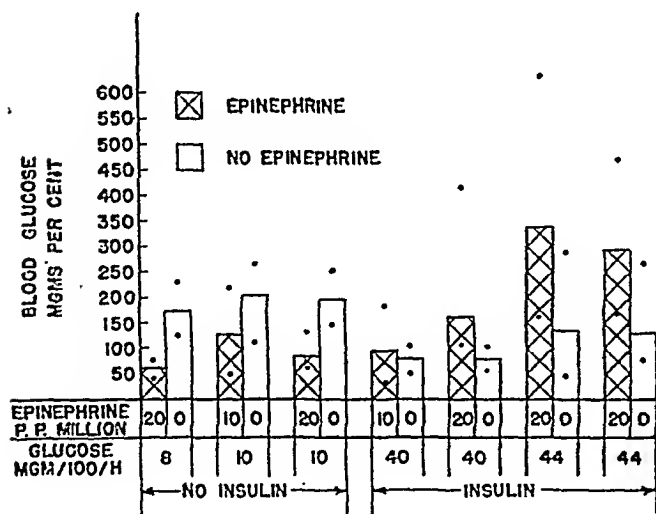


Fig. 2. Effect of epinephrine upon the level of blood glucose at the end of 24 hours of infusion. Averages and range. Twelve rats per group.

with 20 parts per million of epinephrine; *group 2* received 10/100/h. with 10 parts per million of epinephrine and *group 3* received 10/100/h. with 20 parts per million of epinephrine. In each group the level of blood glucose was depressed by epinephrine.

Four groups of rats were given glucose with insulin. *Group 1* was given 40/100/h. with 10 parts per million of epinephrine; *group 2* was given 40/100/h. with 20 parts per million of epinephrine and *groups 3* and *4* each received 44/100/h. with 20 parts per million of epinephrine. In each group there was a significant rise in the level of blood glucose as compared to that of the control animals.

DISCUSSION

Although epinephrine was the first hormone to be isolated in pure form and although its pharmacologic actions have been more fully explored than those of any other hormone, its rôle in physiological processes is still subject to debate. It has been generally believed that the sole effect of epinephrine upon the level of blood glucose is due to stimulation of glycogenolysis in the liver. The results of the present study support the findings of some earlier investigations in showing that under certain conditions epinephrine has an extra-hepatic effect upon glucose tolerance. The conditions of these experiments were abnormal and the amounts of epinephrine administered were large. Therefore, the results may be of pharmacologic rather than physiologic interest.

Bollman, Mann and Magath (3), Soskin (4), Soskin, Priest and Schutz (5), Soskin, Essex, Herrick and Mann (6) found no evidence that epinephrine inhibited the utilization of glucose by the liverless dog. On the other hand, Wilenko (7) reported that epinephrine injections into rabbits inhibited the oxidation of simultaneously injected glucose as judged by the respiratory quotient. Colwell and Bright (8) claimed that epinephrine completely suppressed the oxidation of glucose injected intravenously into cats. Cori (9) presented evidence that epinephrine inhibits the glucose utilization by the muscles in rats. Cori also described one experiment on eviscerated rats given a glucose load of 100/100/h. with added insulin. At the end of one hour those animals which received epinephrine had higher average blood glucose values than did similar animals not receiving epinephrine, although there was considerable overlapping in individual values.

A third result was obtained by Himsworth and Scott (10) using the functionally hepatectomized rabbit. In this preparation epinephrine accelerated the fall in blood glucose, presumably because of its calorogenic action.

In the present experiments the administration of epinephrine without insulin for 2 hours was without effect upon the level of blood glucose but during a longer period epinephrine caused a lowering of the blood glucose level. In the presence of insulin, epinephrine caused a decrease in the tolerance for glucose. Thus we have obtained three different results each of which has been anticipated in the literature. In our studies it would appear that the factors of time and the presence or absence of insulin determine the nature of the effect of epinephrine upon glucose tolerance. It is not apparent that the inconsistencies in the literature can be explained upon this basis. The rabbits studied by Himsworth and Scott (10) had the pancreas intact and were not known to be insulin deficient, yet the administration of epinephrine accelerated the disappearance of blood glucose just as occurred in our insulin-deficient eviscerated rats. A full explanation of the extra-hepatic effect of epinephrine upon carbohydrate metabolism cannot be made from the available data.

SUMMARY

Eviscerated rats were infused intravenously with glucose with and without insulin for periods of 2 and 24 hours. When glucose without insulin was given, the addition of epinephrine did not affect glucose tolerance during a period of 2 hours but during a 24-hour period epinephrine accelerated the rate at which glucose was

removed from the blood. When glucose with insulin was given to similar animals, the addition of epinephrine caused a decrease in glucose tolerance within 2 hours which continued throughout 24 hours.

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WORK PERFORMANCE OF ADRENALLY INSUFFICIENT RATS GIVEN ADRENAL CORTEX EXTRACT BY CONTINUOUS INTRAVENOUS INJECTION

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UP TO the present time we had been unable to sustain a fully normal ability to work in adrenalectomized rats treated with adrenal cortex extracts, steroids or other therapy. In the present study it was found that adrenalectomized rats given large amounts of beef adrenal extract by constant intravenous injection were able to perform as much work as normal and sham-operated rats.

A second purpose of this report is to describe a rapid and simple method of adrenal enucleation without surgery which may have a limited usefulness in acute studies of adrenal insufficiency.

METHODS

Male rats of the Sprague-Dawley strain which weighed 200 ± 2 grams were used. These animals were free from parasites and infections. The diet was Archer Dog Pellets. The procedures used for the stimulation of muscle were according to Ingle (1) with the following modifications. A Nerve Stimulator, Model B, Upjohn, was used to stimulate muscle at the rate of 5 times/second. The duration of each pulse was 20 milliseconds and the intensity was 20 milliamperes. An electrode was placed on the lower tibia of one back leg and the second electrode on the contralateral back foot, thereby activating all of the musculature of both hind legs. The gastrocnemius muscle of the left hind leg was weighted with 100 grams. The distance that the weight was lifted was registered on automatic work recorders. Each recorder revolution represented approximately 400 gram-centimeters of work.

A solution of 0.9 per cent sodium chloride with or without beef adrenal extract (Upjohn) was infused into the jugular vein at a constant rate by means of a constant injection apparatus which delivered fluid from each of 12 syringes at the rate of 20 cc. in 24 hours. The adrenal cortex extract represented 40 gm. of beef adrenal gland per cc. and was free from alcohol. Temperature was constant at $28 \pm 0.5^\circ \text{C}$.

The adrenal glands were removed by the procedure described by Ingle and Griffith (2). In the sham operations the adrenals were exposed but not damaged. The enucleations were done without surgery by palpating the adrenal gland through the body wall and applying gentle pressure with the thumb and forefinger so that the capsule ruptured at a point distal to the pedicle and the gland was extruded from the capsule. A layer of glomerulosa remains adherent to the capsule and is capable of regeneration but during periods up to 48 hours the secretory capacity of this remaining tissue is practically nil. Following enucleation the capsule fills with blood. The loss of blood is almost always slight and is confined to the immediate area of the capsule. The effective application of this procedure requires extensive practice but can be made reliable. The animals were subjected to the work test immediately following operation. Stimulation was continued until the muscle ceased to respond, or for 24 hours.

EXPERIMENTS AND RESULTS

The data of these experiments are summarized in figure 1. Fifteen rats were represented at each dose level and at each control condition. The following doses of

ACE were tested in bilaterally adrenalectomized animals: 0, 12, 16 and 20 cc/24 hours. The following doses of ACE were tested in bilaterally adrenal enucleated animals: 0, 2, 4, 8, 12, 16, 20 cc/24 hours. One group of rats had unilateral adrenalectomies and a similar group had unilateral adrenal enucleations. As controls, one group of normal rats was compared with a similar group having sham operations. Comparisons of adrenalectomized and enucleated rats at the same dosage levels of ACE were carried out at the same times.

The administration of 2 and 4 cc. of ACE per dose did little to increase the work performance of adrenal enucleated rats above that of untreated adrenalectomized and untreated adrenal enucleated animals, but a dose of 8 cc. gave a striking improvement in performance. Doses of 12 and 16 cc. were more effective and 20 cc. restored the performance of adrenalectomized and of adrenal enucleated rats to approximately the same levels as were shown by normal and by sham-operated animals. There was a tendency for the bilaterally adrenal enucleated rats to do

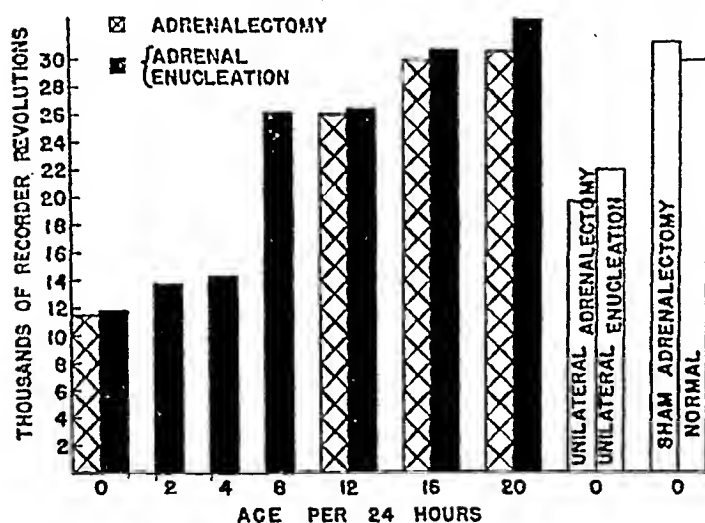


Fig. 1. EFFECT OF ADRENAL CORTEX EXTRACT upon work performance of adrenally insufficient rats. Averages. Fifteen rats per group.

slightly more work than the adrenalectomized rats under comparable conditions but the two series of animals behaved otherwise in a very similar manner. Unilateral adrenalectomy and unilateral enucleation each caused a significant reduction in total work.

DISCUSSION

The experimental conditions of these studies represent a more severe stress than the work test which was standardized as a method of bio-assay (1) and the requirements for ACE are greatly increased. The removal or inactivation of one adrenal gland limits the ability of the rat to work. These data represent further proof that the 'needs' for adrenal cortical hormones are very great during stress and show that the intact adrenal glands can secrete the activity equivalent of very large amounts of ACE.

In an earlier study (3) of adrenalectomized rats under similar conditions the administration of ACE by continuous subcutaneous injection in amounts up to 20 cc. in 24 hours failed to sustain a normal output of work. The continuous intravenous

injection of ACE is thought to simulate, as nearly as possible, the manner in which the adrenal cortices secrete their hormones into the blood. This procedure did enable the adrenalectomized and adrenal enucleated rats to work as well as rats having intact adrenal glands. The possibility remains that the distribution of hormones in beef adrenal extract is not the most optimal mixture for the maintenance of resistance to stress. It is also possible that other substances not contained in ACE would enhance its potency so that the dosage requirement could be reduced.

From these and other data to be published it may be reasonable to conclude that non-surgical adrenal enucleation is a reliable method for producing temporary adrenal insufficiency. The advantages of the speed and simplicity of the method are obvious. Whenever it is desirable to completely inactivate all of the cortical tissue the adrenal glands must be removed surgically.

SUMMARY

A procedure is described for the non-surgical inactivation of the adrenal glands of the rat by palpation of the glands through the body wall and enucleation by gentle pressure.

Adrenalectomized and adrenal enucleated rats were anesthetized with barbiturates and subjected to faradic stimulation of both hind legs at a frequency of 5 times per second. Adrenal cortex extract (ACE) was given by constant intravenous injection during a period of 24 hours. Doses of 2 and 4 cc. of ACE had little effect upon work; 8, 12 and 16 cc. had very striking effects and 20 cc. restored the performance of bilaterally adrenalectomized and of bilaterally adrenal enucleated rats to approximately the levels of normal and sham-operated animals. Unilateral adrenalectomy and unilateral enucleation each caused a marked reduction in total work. There was a tendency for the adrenal enucleated rats to perform slightly more work than the adrenalectomized rats but the relative responses of the 2 series to adrenal inactivation and to therapy were almost identical.

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FAILURE OF ADRENAL CORTICAL HORMONES TO PROTECT AGAINST COLD IN YOUNG NORMAL AND ADRENALECTOMIZED RATS¹

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THE rôle of the adrenal cortex as a protective measure against fall in body-temperature in a cold environment is well known and has been reviewed recently by Swingle and Remington (1). The response to injections of cortical extract has been so consistent that bioassay methods for this extract have been developed by Selye (2) and by Roos (3) in which the degree of protection of rats against exposure to cold is used as a measure of activity. In all such investigations rats 20 days of age or older were used. Recently reported studies by Hill (4) have shown that the albino rat attains its ability to regulate body temperature in a cold environment at about the 18th day of life. It seemed of interest therefore to extend the study of the protective action of adrenal cortical hormones against cold to ages prior to the normal development of body-temperature control. The investigation here reported compares the protective effects of cortical hormones in rats younger than 18 days of age with those in older animals.

METHODS

The rats were exposed in standard, individual, wire nutrition cages in a large cold room (4 x 12 x 7 ft.) at 4° to 7° C. High colonic temperatures were taken by means of an iron-constantan thermocouple and potentiometer as described by Buchanan and Hill (5). The thermocouple was inserted only during the time of taking the temperature.

All comparisons of body-temperature control were made between animals within a litter; no comparisons were made between litters. For example, in a litter of 8 animals, 2 served as controls and 6 were adrenalectomized; 3 of the adrenalectomized rats were given adrenal cortical extract (cortin) during the experiment.

The efficiency of body-temperature control in a cold environment was tested on normal animals, bilaterally adrenalectomized animals, and animals in which bilateral autotransplantations of the adrenal glands were made into the superficial fascia of the thigh over the femoral vessels. The adrenalectomies and transplantations were performed by a technique similar to that of Griffith and Farris (6). All operations were done under pentothal sodium anesthesia on the day preceding the cold stress. Animals under 18 days of age were returned to their mothers over night following the operations. Sham-operated animals were anesthetized and subjected to all surgical procedures in the same manner as the adrenalectomized and gland-transplanted animals except that the adrenal glands were not disturbed.

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Upjohn's adrenal cortical extract, which is in 10 per cent alcoholic solution, was used throughout the experiments and was given in two equally divided doses. It was administered subcutaneously in the hip region, near the base of the tail, unless otherwise stated. Doses were varied according to the ages and weights of the animals: 29- to 30-day-old rats were given two equal doses each of 0.3 to 0.6 cc.; 15- to 18-day-old rats, two doses of 0.3 cc.; 10- to 13-day-old rats, two doses of 0.15 to 0.20 cc.; and 8-day-old rats, two doses of 0.10 to 0.15 cc.

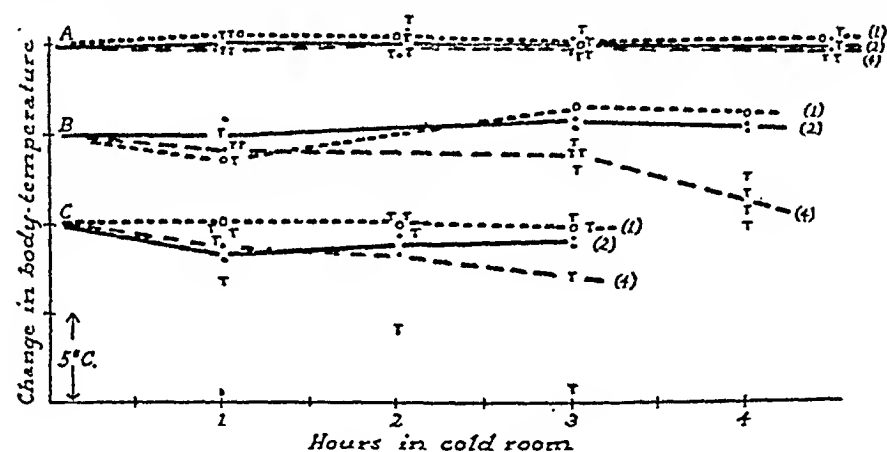
In an attempt to evaluate the possible rôle of the nervous connections of adrenal glands in the response of the young animal to a cold environment, the experiments which utilized bilateral transplantation of the glands were performed. The animals in this experiment were 18 days old on the day of transplantation, and their resistance to exposure to a cold environment was measured on the same day a few hours before the operation. Following the transplantation, they were given maintenance doses of cortin for 5 days. On the 13th and the 21st day following the transplantation, they were again subjected to the cold environment. After the last subjection to cold, the success of the transplantation of the adrenal glands was determined either by gross and microscopic observation of the transplantation site and the internal organs of the animal at the time of autopsy, or by subjection of the animal to a test of adrenal insufficiency, or by both. The test of adrenal insufficiency consisted in the observation of survival time after removal of the transplanted adrenal from its location.

In all, 222 albino rats were subjected to stress in the cold room. The ages of the rats were 8, 10 to 11, 12 to 13, 15, 16, 18, and 29 to 30 days. The animals within each age group were subjected to different experimental procedures and compared with the normals. The different experimental procedures whose effects were studied consisted of adrenalectomy with and without cortin, sham operation with and without cortin, and the administration of cortin to the normal unoperated rat. When administered, cortin was given at various time intervals before and during the subjection to cold as is discussed later and as is shown in the charts.

RESULTS AND DISCUSSION

Nervous connections to the adrenal glands were found not to be essential for maintenance of body-temperature. One litter of 7 rats, 18 days of age, was subjected to cold stress for 4 hours; the results are illustrated in fig. 1A, where, as in all the other charts, the curves represent the mean values of the individual determinations and the numbers in parentheses at the right of the curve indicate the number of rats represented by the curve. During this first subjection to cold, all 7 animals were able to maintain their normal body-temperatures. A few hours after the cold stress, 4 rats were subjected to autotransplantation of the adrenal glands, and one rat was subjected to a sham operation, leaving 2 normal unoperated rats as controls. When this same group of 7 rats was subjected to cold stress a second time, 13 days later (fig. 1B), the normal and sham-operated rats maintained normal body-temperature, but the animals with the transplanted adrenals showed a 1° to 3°C. drop in body-temperature in 3 hours and a 3° to 5°C. drop at the end of 4 hours. Eight days after this (fig. 1C), the same group was subjected to cold stress a third time, but for only 3 hours. The normal, sham-operated, and 3 of the 4 animals with the

transplanted adrenals maintained their normal body-temperatures, but the 4th animal with the transplanted adrenal showed a 9°C . drop in 3 hours. This animal



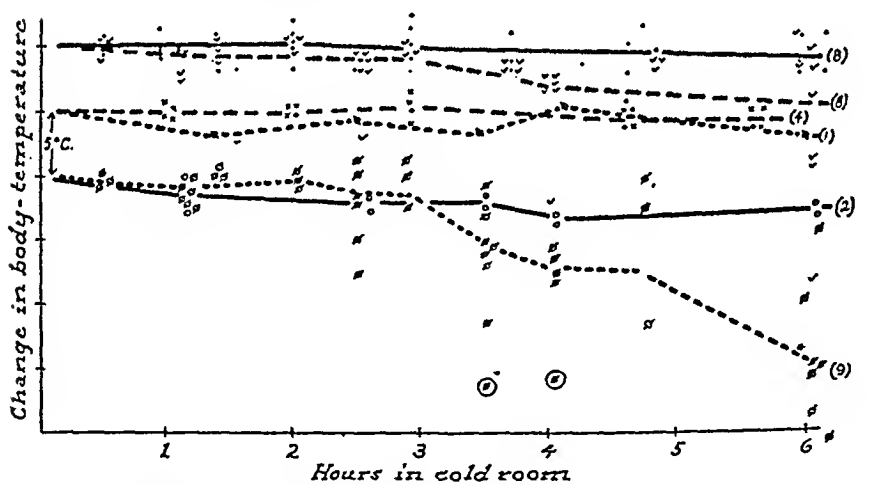
• Normal
 ○ Adrenal transplantation on 18th day of life
 × Sham operation on same day

A—Just before operation

B—13 days after operation

C—21 days after operation

Numbers in parentheses indicate number of rats used



• Normal
 ○ Normal + cortin, 3 hours before and just before stress
 × Adrenalectomy + cortin, 3 hours before and just before stress
 □ Sham operation + cortin, 3 hours before and just before stress
 • Adrenalectomy
 *—Includes 2 estimated values
 ⊙—Removed from cold room after this del'n.
 Numbers in parentheses indicate number of rats used

Fig. 1 (upper). BODY-TEMPERATURE REGULATION in 1 litter of 7 rats. Adrenal transplanted, sham-operated and normal animals.

Fig. 2 (lower). BODY-TEMPERATURE REGULATION in 4 litters of 32 rats 29-30 days of age.

showed anal bleeding during the time of the last subjection to cold stress. The body-temperature of this animal may have fallen because of accidental mechanical injury incurred in taking the colonic temperature, or because of lack of adrenal tissue. It died a few days after this third exposure to cold. Two of the animals

with transplanted adrenals were killed 10 days after the last subjection to cold stress and, at autopsy, both showed good growths of the adrenal glands at the site of the transplantation. Neither showed any grossly observable adrenal tissue in the normal gland site, in the perirenal fat or anywhere in the abdominal cavity. Microscopically, both showed survival of cortical adrenal tissue at the site of transplantation with sufficient distortion of architecture to indicate regeneration of cells and multiplication of vascular channels. The insufficiently vascularized medullary portion showed complete necrosis. The 4th animal with the transplanted adrenal was subjected to the physiological test of adrenal insufficiency rather than to autopsy observation alone. This rat was anesthetized 12 days after the last exposure to cold and the adrenal glands were removed from the site of transplantation. Grossly and microscopically, the transplants showed survival of cortical adrenal tissue. The animal died 17 days later. Observation at autopsy detected no adrenal tissue in the animal, either grossly or microscopically.

Another litter of 5 animals of the same age (the data of which are not presented in a chart) was subjected to the same body-temperature reaction studies. Three of these 5 rats had the adrenal glands transplanted, one was sham-operated, and one was a control. The entire litter showed good maintenance of body-temperature in the cold environment and 3 of the animals with transplanted adrenals maintained their body-temperature during the third subjection to cold. Twelve days after the last exposure, the transplanted adrenals were removed and 2 of the animals died within 11 days. At autopsy, these animals showed no grossly recognizable adrenal tissue. Microscopic studies were not made on these 2 rats. The third lived 40 days and was then killed. Although gross inspection at autopsy showed what might have been diffuse adrenal tissue in the fat near the site of transplantation, no cortical cells could be detected microscopically. However, a nest of cells consistent with cortical cells was observed in microscopic section of the perirenal fat.

It was necessary to test the conformity of our animals to the previously reported reactions to cold stress (2, 3). That they did conform is shown by the results obtained with 29- and 30-day-old rats (fig. 2). Four litters of animals (32 rats) of these ages were subjected to cold for 6 hours, or, in those which failed to regulate, until the body-temperatures had fallen 20°C. Good maintenance of body-temperature, with little individual variation, was observed in the following groups: 8 normal unoperated rats; 4 normal unoperated rats that received cortin 3 hours before and just before stress; 2 sham-operated animals; and one sham-operated animal that also received cortin 3 hours before and just before stress. However, the group of adrenalectomized animals, both with and without cortin, showed great variations. Nine adrenalectomized rats showed decreases in body-temperature within 6 hours. One showed a drop of 17° C. in 3½ hours, another 16° C. in 4 hours, but one showed only a 3° C. drop in 6 hours. This last reaction may have been the result of incomplete adrenalectomy. Since the body-temperatures of 2 animals had dropped more than 20° C. before the end of 6 hours, their probable body-temperatures at the 6th hour were estimated. Taking into consideration these 2 animals and the 7 animals which remained in the cold room for 6 hours, an approximate average temperature fall of about 15° C. in 6 hours occurred in this group of adrenalectomized rats which did not receive cortin.

Eight adrenalectomized rats received cortin 3 hours before and just before stress. The body-temperature of one began to decrease within one hour and showed a decrease of 18°C . in 6 hours. The other 7 maintained their body-temperatures well for 3 hours and then showed a slow decrease. The maximum loss of body-temperature of any one of these 7 animals was 9°C . in 6 hours and the average loss for the 7 animals was 3.6°C . in 6 hours. This drop in body-temperature shows good regulation as compared with the average body-temperature loss of 15°C . in 6 hours in the adrenalectomized rats that did not receive cortin. This set of experiments shows that the adrenal cortical hormones protected these 29- and 30-day-old animals against exposure to cold.

Considerably different results were obtained with one litter of 5 animals 18 days of age (fig. 3). Good maintenance of body-temperature was accomplished by one normal unoperated rat during 4 hours in the cold room. Two adrenalectomized litter mates that did not receive cortin showed temperature decreases of 19°C . in less than 3 hours and 17°C . in $3\frac{1}{2}$ hours, respectively. The response to cold of the 2 remaining adrenalectomized litter mates that received cortin 3 hours before and just before stress varied markedly. One showed good body-temperature regulation with a drop of only 5°C . in 4 hours of stress. The other showed a drop of 20°C in $3\frac{1}{2}$ hours. This variability of body-temperature regulation at 18 days of age is consistent with the transition period of regulation in normal rats as described by Hill (4).

That this transition period may begin as early as the 16th day of life is shown by the results obtained with a litter of 7 unoperated animals (fig. 4). Four rats were injected intraperitoneally with cortin one hour before cold stress (fig. 4A) and compared with 3 litter-mate controls (fig. 4B). At the end of 3 hours in the cold room, the body-temperature of 4 cortin-injected rats had decreased approximately 2.5° , 6° , 8° , and 10°C ., respectively, and by the end of 4 hours had fallen about 3.5° , 9° , 13° , and 22°C . In comparison, the 3 uninjected controls had decreased 2.5° , 6.5° , and 22°C ., respectively, in 3 hours. The one showing the 22°C . decrease was removed from the cold room at this time. The 2 remaining rats showed a final decrease of 4° and 17°C . in body-temperature in 4 hours. When the estimated 4-hour body-temperature of the rat which had been removed from the cold room at the 3rd hour was averaged with the other 2 final temperatures, the average fall in the 3 control animals was about 16°C . This is comparable to the average temperature drop of 12°C . demonstrated by the 4 animals which received cortin. Since the 16-day-old group is represented by so few animals, the small differences may or may not be significant, but there is certainly an indication that in some litters the transition period may begin at about this age (4).

In a litter of 7 rats 15 days of age (fig. 4C and D), 4 unoperated rats injected with cortin 17 hours before and 1 hour before stress reacted to cold in a manner similar to their uninjected litter mates. At the end of the stress period ($1\frac{1}{2}$ hours) the average fall in body-temperature of the injected group was about 13°C ., and that of the uninjected group about 15°C . Whereas the cortin may have had some slight effect, it did not enable the injected animals to maintain their body-temperatures.

In the 29 and 30 day old rats cortical hormones, whether normally present or injected into the adrenalectomized animal, showed a definitely positive protective effect against decrease in body-temperature when the rats were exposed to cold.

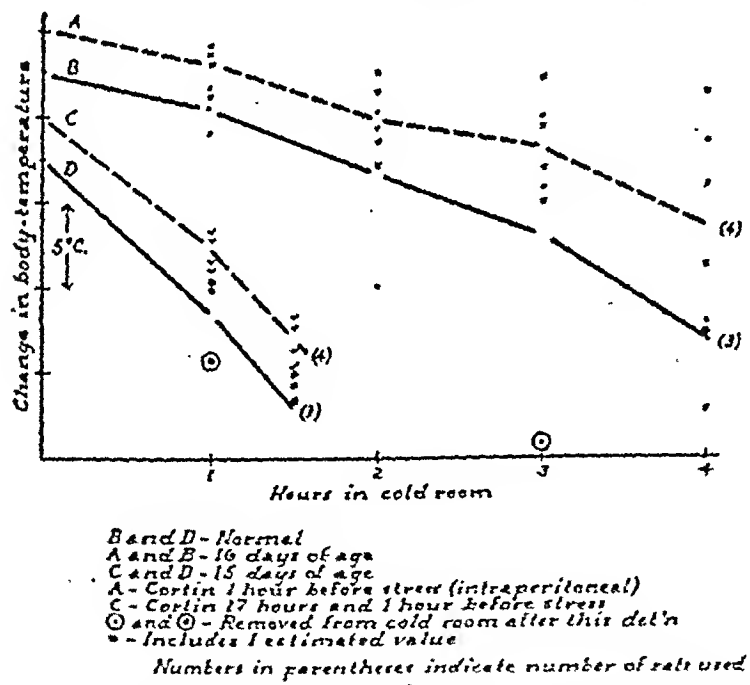
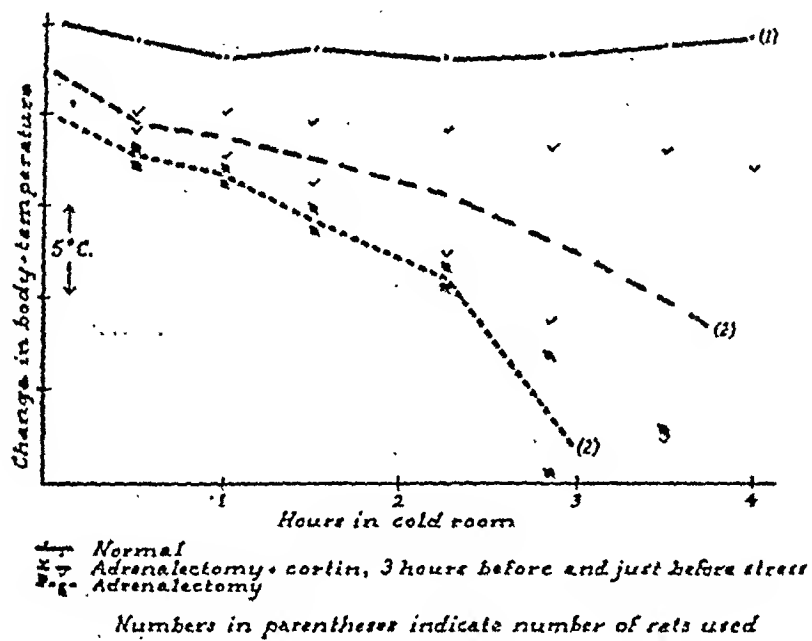
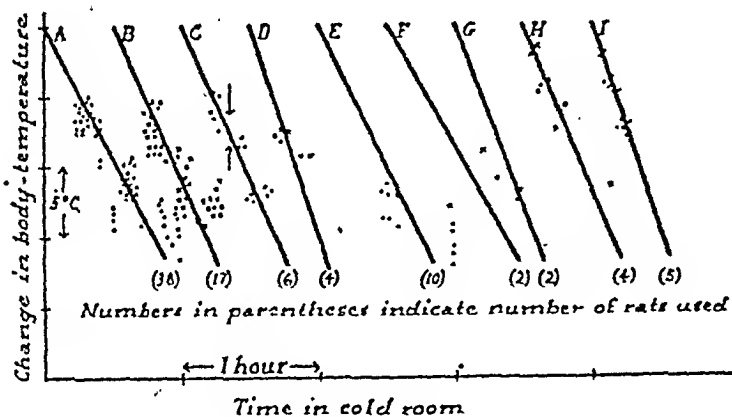


Fig. 3 (upper). BODY-TEMPERATURE REGULATION in 1 litter of 5 rats 18 days of age.

Fig. 4 (lower). BODY-TEMPERATURE REGULATION in 2 litters of 14 rats 15 and 16 days of age.

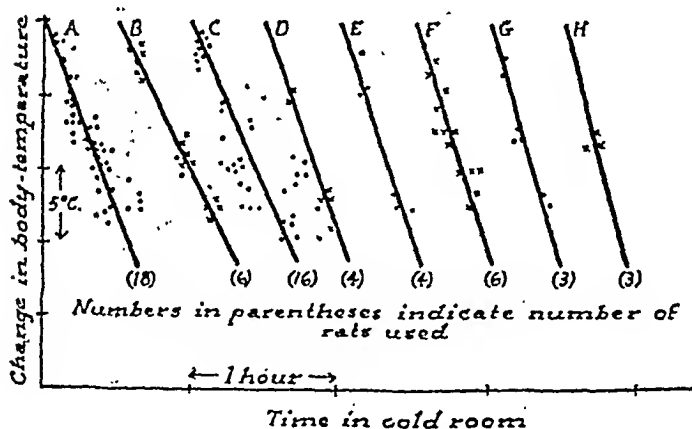
This effect was not constant in the 18, 16 and 15 day old rats. In rats younger than this, the cortical hormones had absolutely no protective effect against decrease in body-temperature as was demonstrated in all experiments on rats less than 15 days of age.

Animals 13 days old and younger decreased in body-temperature more rapidly than the older ones, so that they were removed from the cold room in less than 1 hour. Many of these young animals, especially those adrenalectomized and sham-



- A-Normal
 B-Normal + cortin 3 hours before and just before stress
 C-Normal + cortin 3 hours before, just before and during stress
 D-Normal + cortin 1 hour before and just before stress
 E-Normal + cortin 1.7 hours before and 1 hour before stress
 F-Sham operation
 G-Sham operation + cortin 3 hours before and just before stress
 H-Adrenalectomy
 I-Adrenalectomy + cortin 3 hours before and just before stress

Arrows in third curve show time of cortin administration



- A-Normal
 B-Normal + 10% ethyl alcohol in saline just before stress
 C-Normal + cortin 3 hours before and just before stress
 D-Sham operation
 E-Sham operation + cortin 3 hours before and just before stress
 F-Adrenalectomy
 G-Adrenalectomy + cortin 3 hours before and just before stress
 H-Adrenalectomy + cortin 1 hour before and just before stress

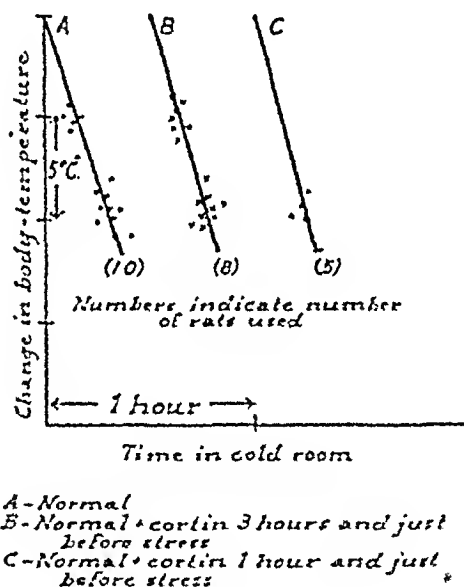
Fig. 5 (upper). BODY-TEMPERATURE REGULATION in 12 litters of 88 rats 12 and 13 days of age.
 Fig. 6 (lower). BODY-TEMPERATURE REGULATION in 10 litters of 60 rats 10 and 11 days of age.

operated, had an original temperature below that of the usual 37° C. and for that reason they were removed from the cold room when their body-temperatures fell below the level of 17° to 20° C. This was in contrast to the 29- and 39-day-old group

which were removed from the cold room when their body-temperatures had fallen 20°C . below the original value.

Body-temperature regulation was studied in 88 rats 12 and 13 days of age. Thirty-eight rats were used to establish the efficiency of body-temperature regulation in a cold environment by normal rats of that age (fig. 5A). The values of the individual determinations are somewhat scattered on both sides of the curve representing the mean, but approximately the same type of scatter is observed on a parallel curve (fig. 5B) obtained from the mean value of the individual determinations of body-temperature of 17 unoperated rats which received cortin 3 hours before and just before stress. To determine whether larger doses of cortin would modify the results, a third injection of cortin was given after 20 minutes of cold stress to 6 rats which had received the drug 3 hours before and just before stress (fig. 5C) in the same manner as the former group (fig. 5B). They showed no better temperature

Fig. 7. BODY-TEMPERATURE REGULATION in 3 litters of 23 rats 8 days of age.



regulation than either of the other groups. Furthermore, administration of cortin 17 hours before and one hour before cold stress (fig. 5E), or one hour before and just before stress (fig. 5D), also failed to prevent a temperature drop similar to that observed in the uninjected animals. Sham-operated animals with (fig. 5G) or without (fig. 5F) cortin showed a mean body-temperature drop parallel to that of the untreated controls. Adrenalectomized rats (fig. 5H) did not decrease in body-temperature any more rapidly than did the normals. Adrenalectomized rats that received cortin (fig. 5I) did not decrease in body-temperature any less rapidly than did the adrenalectomized rats that did not receive cortin. Whether normal, sham-operated or adrenalectomized, and whether or not cortin was given, all rats 12 and 13 days of age showed a decrease in body-temperature of approximately the same rate when exposed to a cold environment. This is in contrast to the reactions of 29- and 30-day-old animals, in which cortical hormones altered the response to cold.

The decrease in body-temperature of the 10- and 11-day-old rats, when exposed

to a cold environment, was more rapid than that of the 12- and 13-day-old rats (fig. 6) which can probably be explained by the difference in the size of the animals (4). The extent of individual variations of decrease in body-temperature from the mean values is about the same in the two age groups. Results with both age groups (figs. 5 and 6) emphasize the fact that cortical hormones have no protective effects against decrease in body-temperature in rats younger than 16 days of age.

Since the cortin was in a 10 per cent alcoholic solution, the possible caloric effect of the alcohol was tested on 6 rats, 10 and 11 days of age (fig. 6B), by the parenteral injection of isotonic saline containing 10 per cent alcohol. The results show that it is unlikely that alcohol in any way alters the rate of decrease in body-temperature in rats of this age.

Since the administration of cortin, 3 hours before and just before stress, to 10- and 11-day-old adrenalectomized rats did not give a protective effect against fall in body-temperature (fig. 6G) as compared with untreated adrenalectomized rats (fig. 6F), a shorter time interval between injection and exposure was tested. When cortin was given one hour before and just before stress the response to cold was not altered.

This same approach was used in 8-day-old rats (fig. 7) in which cortin was administered at two different time intervals to unoperated rats. The average rate of decrease in body-temperature is the same in all cases whether no cortin was administered (fig. 7A), whether cortin was administered 3 hours before (fig. 7B), or whether cortin was administered one hour before and just before stress (fig. 7C).

The reasons for the failure of adrenal cortical hormones to protect against decrease in body-temperature in rats younger than 16 days of age are still unknown. These experiments suggest that the action may be mediated through some mechanism not yet developed in the young animal. It is possible that this mechanism is enzymatic in nature. Studies to test this hypothesis are now under way in this laboratory.

SUMMARY

The adrenal cortical hormones offer no protection against decrease in body-temperature in rats under 16 days of age when exposed to a cold environment. This is in contrast to the effective protection provided by these hormones in older rats. These experiments give strong evidence that the adrenal cortical hormones may function in protection against cold through a mechanism that is not developed before the 16th day of age.

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ANTITHYROID ACTIVITY OF ERGOTHIONEINE

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THE recent papers of Lawson and Rimington (1) and Astwood and Stanley (2) presenting conflicting data on the antithyroid activity of ergothioneine, a naturally occurring constituent of blood, aroused our interest in evaluating this compound for antithyroid potency by the technics used in our laboratory. Further incentive was provided by the observations of Latner and Mobray (3) who found that the fasting red blood cell ergothioneine was lower than normal in four patients with thyrotoxicosis and low normal in two others. Pitt-Rivers (4) has observed recently that ergothioneine inhibits the conversion of acetyldiiodotyrosine to acetyl-thyroxine *in vitro*.

The experiments of Lawson and Rimington on rats indicated that ergothioneine possessed an antithyroid activity essentially equal to that of thiouracil, a not unexpected finding in view of the potency of many mercaptoimidazole derivatives (5-7). However, in man and in rats as well, Stanley and Astwood found that ergothioneine in substantially large doses was completely inactive. Our data, in agreement with those of Stanley and Astwood, show that ergothioneine is not antithyroid in the monkey and rat.

PROCEDURE AND RESULTS

Chronic Feeding Experiments in Rats. The technic used was identical with that described in an earlier publication (8). Immature rats of 40 to 50 grams initial weight were given ergothioneine mixed with their diet for a period of 10 days. Thyroids were removed, weighed and analyzed for iodine content. Results are shown in table 1 which includes for comparison thyroid weight and iodine concentration of untreated and of thiouracil-fed control rats.

It will be observed from these results that ergothioneine has no antithyroid activity in the doses used. These negative findings agree with those of Stanley and Astwood (2) who fed young rats a diet containing 0.03 per cent ergothioneine or injected them with 5 mg. daily of the drug for 10 days.

Radioiodine Absorption by Rat Thyroids. The method is based on the inhibitory effect of antithyroid compounds on the natural capacity of the thyroid to concentrate iodine for hormone synthesis. Details of the procedure used were described by McGinty *et al.* (9) for evaluation of certain goitrogens using the single injection technic. Ergothioneine was injected subcutaneously into groups of 140 to 150-gram rats. After one hour, radioiodide was injected intraperitoneally. Four hours after radioiodide administration, thyroids were removed, digested in KOH solution and

the radioactivity measured on aliquots of the digest and on samples of the radioiodide injected. In each experiment the percentage of radioiodide collected by the thyroid is calculated from these measurements and compared with that collected by the thyroids of untreated controls given radioiodide at the same time. In table 2 these values are presented as ratios and also as percentage of control uptake.

It will be observed that 8 mg. of ergothioneine exerted no block to iodine collection by the thyroid gland whereas thiouracil was fully effective at .5 mg. and partially effective at .25-mg. doses.

TABLE 1

DRUG	CONC. IN FOOD	DAILY INTAKE OF DRUG/100-GM. RAT	THYROID WT. MG/100-GM. RAT	THYROID IODINE CONC.
	%	mg.		mg. %
Ergothioneine HCl · 2H ₂ O.....	.06	6.7	8.5	51.2
	.03	3.6	8.7	66.0
	.01	1.2	9.8	58.4
Thiouracil	.03	3.2	24.0	2.0
	.01	1.1	14.5	7.0
Untreated controls			7.1	62.0

3 rats per group. Thyroids pooled for iodine analysis.

TABLE 2

DRUG	DOSE, MG.	RATIO % RaI COLLECTION-TREATED TO CONTROL RATS	% UPTAKE RaI
Ergothioneine ¹	8	11.1 ± 1.7 / 11.5 ± 1.4	96.5
	4	13.1 ± 1.5 / 11.5 ± 1.4	114.0
	2	10.4 ± 0.4 / 11.5 ± 1.4	90.3
	1	12.4 ± 1.0 / 11.5 ± 1.4	108.0
Thiouracil ¹	1	0.7 ± 0.1 / 11.0 ± 0.2	6.4
	0.5	2.1 ± 0.3 / 30.5 ± 1.7	6.9
	0.25	6.2 ± 0.7 / 24.7 ± 3.3	25.1

¹9 rats per group.

Radioiodine Uptake in Monkeys. The technic employed was adapted from that used by Stanley and Astwood (10) for evaluating antithyroid drugs in human subjects and described by them in detail. At the start of the experiment, 4 to 7-kg. monkeys were injected intravenously with 20 microcuries I¹³¹. Beginning shortly thereafter, radioactivity counts were recorded over the neck region at intervals up to 24 hours. Ergothioneine was administered either orally in a capsule or intravenously in saline solution. In parallel experiments, thiouracil was given orally. In all cases, the drugs were administered 3 hours following radioiodide injection at which time recorded neck counts plotted against the square root of the time in minutes had established an 'accumulation gradient' characteristically linear for at least 12 hours.

Effectiveness of an active antithyroid substance may be estimated from deviations from the slope of the accumulation gradient.

Figure 1 shows the results of four experiments. The lower two curves demonstrate the deviation from the accumulation gradient resulting from two doses of thiouracil, one moderately effective and the other fully effective, along with control curves on the same animals. The upper two curves show the results of large doses of ergothioneine administered orally and intravenously and their respective controls. It will be observed from these curves that ergothioneine in the doses indicated was ineffective in altering the rate of iodine absorption by the thyroid and therefore exerted no effect on the hormone synthesis mechanism. Thiouracil, on the contrary

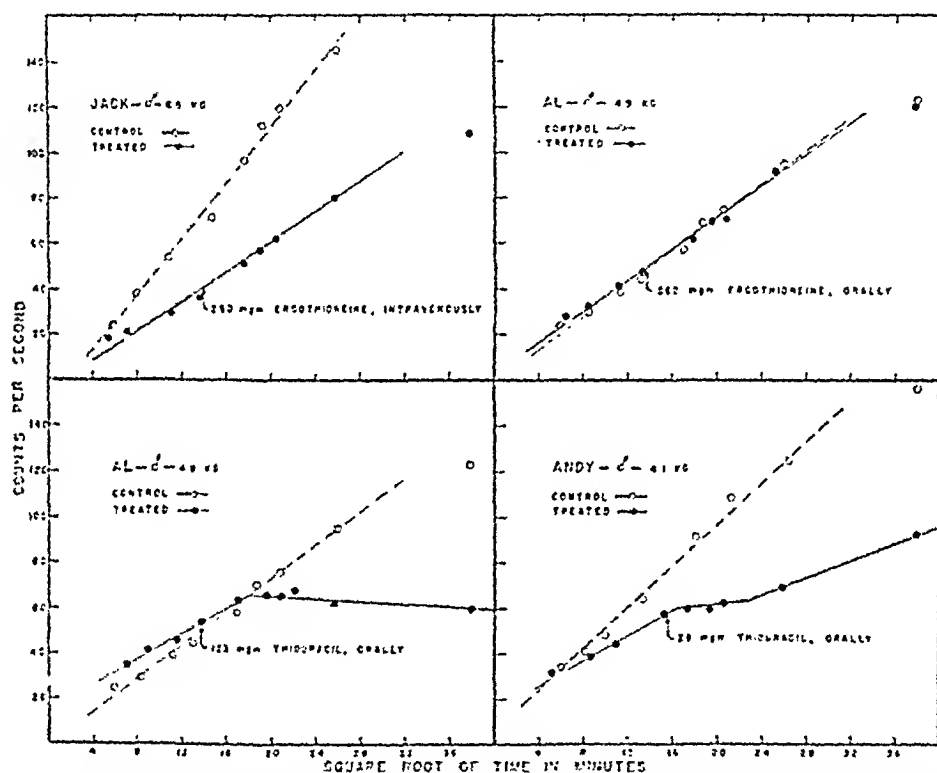


Fig. 1. COMPARISON OF ACTION of ergothioneine and thiouracil on the rate of radioiodine absorption by the thyroid gland of the monkey.

in a dose as small as 25 mg. (6 mg/kg.) interfered with iodine utilization beginning shortly after administration of the drug and persisting for approximately 4 hours when the upward trend of the absorption curve indicated resumption of hormone synthesis. In a dose of 123 mg. (25 mg/kg.) thiouracil was effective for at least 24 hours.

DISCUSSION

In their discussion of Astwood and Stanley's paper, Lawson and Rimington (1) point out that ergothioneine injected intramuscularly or subcutaneously in daily doses of 100 mg. failed to bring about any reduction in the basal metabolic rate of two thyrotoxic patients, one of whom was treated for 17 days. They suggest on the basis of ergothioneine urinary excretion studies on normal persons, that this dose

may be wholly inadequate. Whether inactivity is due to rapid metabolic destruction of the compound or to rapid excretion is not known although Latner and Mowbray have suggested that the latter is the more likely explanation.

Examination of available biological data in the rat (7) on 2-mercaptoimidazole and its 4-alkyl derivatives reveals a wide range of antithyroid activity even among closely related compounds. Thus, 4-n-propyl-2-mercaptoimidazole exhibited an activity 4-5 times that of thiouracil and some 3 times that of the parent compound, while the 4-n-amyl derivative was virtually inactive. Similarly, 4-cyclohexylmethyl-2-mercaptoimidazole showed no activity whereas its aromatic analog benzyl-2-mercaptoimidazole was at least as active as thiouracil. Ergothioneine, the methyl betaine of mercaptoimidazole, may well belong to the inactive group of compounds.

SUMMARY

Ergothioneine in doses substantially larger than effective amounts of thiouracil exhibited no antithyroid activity in the rat and monkey.

The ergothioneine hydrochloride dihydrate used in the experiments was kindly supplied by Dr. J. J. Pfüfner of the laboratory of Parke, Davis and Company.

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ORAL EFFECTIVENESS OF D,L-THYROXINE IN CRYSTALLINE, MONOSODIUM, AND DISODIUM FORMS¹

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IN VIEW of the obvious importance of the thyroid hormone in clinical therapy and its effect on the productive processes of livestock, the problem of the absorption of thyroxine from the gastrointestinal tract is of great interest. Apparently the oral efficacy of thyroxine depends a great deal on the form in which the hormone is administered. This fact is shown clearly by studies on urinary iodine excretion (1, 2) and the calorogenic effects of various thyroxine compounds (3).

It is an elementary chemical fact that thyroxine is virtually insoluble in water but can be dissolved if the solution is made markedly alkaline. It seems logical to assume, therefore, that dissolved thyroxine or the more soluble forms of thyroxine should be more easily assimilated. A perusal of the literature on this subject indicates that this expectation seems to be justified.

Thus, Schittenhelm and Eisler (4) found that when thyroxine was introduced in alkaline solution into the isolated intestine of the dog, 90 per cent of the iodine thus administered was absorbed in from 2 to 12 hours. On the other hand, the same investigators found that, in man, after the administration of the same amount of thyroxine in crystalline form, they could recover 86 per cent of the administered iodine in the feces. At best, then, only 14 per cent was absorbed.

Likewise, Elmer and Rychlik (2) observed the urinary iodine elimination in man after the oral administration of thyroxine, both crystalline and in alkaline solution. When pure crystalline thyroxine was given *per os*, only 6 per cent of the introduced iodine could be recovered in the urine in 24 hours. On the other hand, after the administration of the same amount of thyroxine (1300 γ I) in alkaline solution, 14 per cent of the iodine was excreted in the urine in 24 hours. An equivalent dose of potassium iodide resulted in the elimination of 31 per cent of the introduced iodine. These observations bear out, in substance, the results of other investigations on urinary iodine elimination (1, 5, 6). Elmer (7), in his book on iodine metabolism, also pointed out that the rapidity of increase and decrease in blood iodine depends on the form of thyroxine used, solubility apparently being an important factor.

The above experiments show unmistakably that the absorption of thyroxine, as indicated by the urinary iodine excretion, depends to a large extent on the solubility of the thyroxine compound administered. It should be noted, however, that the diminished absorption of thyroxine as compared to that of potassium iodide is apparently not the only reason for less iodine elimination. Bue and Elmer (1) found that even after thyroxine is injected intravenously the urinary iodine excretion is both smaller and longer delayed than after the injection of the same amount of iodine in the form of potassium iodide.

Thompson, *et al.* (3) used a different approach to the problem of determining the oral efficacy of thyroxine and its salts. They studied the effects of these forms of thyroxine on the basal metab-

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olism of three myxedematous patients. On this basis it was found that the disodium salt (thyroxine in alkaline solution) was the most effective. Monosodium thyroxine tablets were found to be one third as active as the disodium form; and pure crystalline thyroxine contained only one thirty-fifth the activity of the monosodium salt. These workers, too, concluded that the solubility of the compound administered was the most important factor in the absorption of thyroxine from the gastrointestinal tract.

It seemed worthwhile to the authors to pursue this study further especially since a method is now available which, we believe, would give a more accurate indication of the relative activity of the various thyroxine compounds. Also, it seemed that if solubility is the important factor a more valid comparison would be obtained by administering all three forms of thyroxine in the same physical state, i.e. in solid form as contrasted, for example, with feeding the disodium salt already in solution. It was with these considerations in mind that the present study was undertaken.

METHODS AND MATERIALS

The technique used in these experiments for determining the activity of the thyroxine compounds was essentially the same as that proposed by Dempsey and Astwood (8) for measuring the thyroid secretion rate. In general, this technique embodies the hypothesis that the amount of thyroxine necessary to counteract the effects of thiouracil (compensatory hypertrophy) and maintain the thyroid gland at normal weight is equivalent to the amount of hormone normally produced by the thyroid. It was thought that, for our experiments, this method could be used to advantage. By including crystalline, monosodium and disodium thyroxine in the feed of thiouracil-treated chicks, the amount of each form required to maintain thyroid glands at normal weight could be ascertained. Thereby, we should obtain a fairly accurate measure of the relative oral effectiveness of these thyroxine compounds.

The chicks used in these experiments were day-old White Plymouth Rocks purchased from a local hatchery. As they were obtained the chicks were split into various groups. One group served as a control; each other group received 0.1 per cent thiouracil and one of the various doses of the form of thyroxine then under consideration. In the preliminary attempts fairly wide dosage intervals were used. The results of these preliminary attempts are not reported, since they were intended primarily for the orientation of the proper dosage ranges. When the proper range had been determined, the dosage interval was narrowed. In order that we might feel assured that the proper dosage levels had been attained, the latter runs were repeated. The results reported in table 1 and figures 1 and 2 represent a combination of the data obtained in these two runs, with the exception of the groups receiving 0.000030 per cent by weight of monosodium and disodium thyroxine, where only one was conducted.

Each experiment was of three weeks' duration. At the end of this period the chicks were killed, sexed, and their body weights determined. The thyroid glands were removed and weighed immediately.

It should be mentioned that some difficulty was encountered in determining the dosage range and interval for the chicks receiving crystalline thyroxine. For some

inexplicable reason, one or two of the earlier attempts indicated dosages misleadingly high. However, several successive attempts yielded reasonably repeatable results. It was found, however, that it was impossible to narrow the dosage interval to the same extent as with the salts of thyroxine, due to the fact that the variability of thyroid weights within dosage groups confused the interpretation of the data.

TABLE 1. EFFECT OF D,L-THYROXINE AND ITS SALTS IN THE FEED OF THIOURACIL-TREATED CHICKS (WHITE PLYMOUTH ROCK 3-WEEK-OLD CHICKS)

FORM OF THYROXINE	DOSAGE	CORRECTED DOSAGE	NO. OF CHICKS	BODY WT.	THYROID WT.	THYROID WT./100 GM. BODY WT.	ESTIMATED NORMAL REQUIREMENT
<i>Males</i>							
Control ¹	<i>per cent</i>	<i>per cent</i>		<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	<i>per cent</i>
Crystalline	0.000050	0.000050	48	160.1	8.9	5.6	
	0.000075	0.000075	26	145.2	23.1	15.9	
	0.000100	0.000100	21	173.1	8.9	5.1	0.000074
	0.000100	0.000100	23	162.6	4.6	2.8	
Monosodium	0.000025	0.0000243	18	161.9	33.5	20.7	
	0.000030	0.0000291	7	141.0	15.4	10.9	
	0.000035	0.0000340	31	153.7	9.7	6.0	
	0.000040	0.0000388	20	163.5	5.2	3.2	0.000035
Disodium	0.000025	0.0000236	24	161.7	34.5	21.3	
	0.000030	0.0000283	7	133.7	21.7	20.3	
	0.000035	0.0000330	33	150.5	13.3	8.8	
	0.000040	0.0000378	16	169.3	9.6	5.6	0.000038
<i>Females</i>							
Control ¹			47	148.2	9.2	6.2	
Crystalline	0.000050	0.000050	15	151.7	18.6	12.3	
	0.000075	0.000075	18	161.6	10.9	6.7	
	0.000100	0.000100	14	156.1	4.8	3.1	0.000078
Monosodium	0.000025	0.0000248	18	156.9	43.1	27.5	
	0.000030	0.0000291	11	139.5	22.7	16.3	
	0.000035	0.0000340	28	147.8	7.9	5.3	
	0.000040	0.0000388	19	146.6	6.6	4.5	0.000036
Disodium	0.000025	0.0000236	15	140.8	42.6	30.3	
	0.000030	0.0000283	12	121.5	25.3	20.9	
	0.000035	0.0000330	28	143.3	16.6	11.6	
	0.000040	0.0000378	22	151.7	10.9	7.2	0.000039

¹ All groups except controls received 0.1% thiouracil in their feed.

The crystalline D,L-thyroxine used was a commercial preparation purchased from the British Drug House, London. The monosodium and disodium salts were made from this stock by the method outlined by Harington (9). These salts were checked for purity by iodine analyses, which showed that the monosodium salt contained 63.70 per cent I and the disodium salt, 60.91 per cent, as compared to theoretical percentages of 63.46 per cent and 61.69 per cent, respectively.

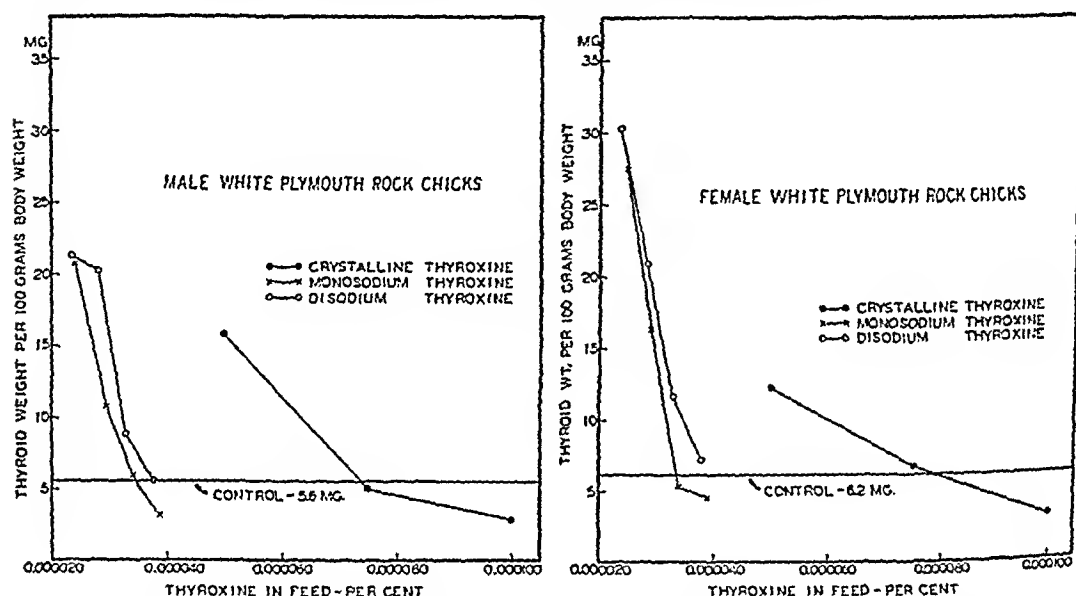
Both the thiouracil² and thyroxine were mixed in the feed as the proper per-

² The authors thank Lederle Laboratories, Pearl River, N. Y., for supplying the thiouracil used in these experiments.

centage by weight. In this way, of course, a certain error was introduced. The addition of sodium atoms to the thyroxine molecule causes a diminution of the actual amount of thyroxine received by the groups treated with thyroxine salts. This error is taken into account in the presentation of the data (table 1; figs. 1 and 2).

RESULTS

It was found that the requirements for maintaining the thyroid glands of the thiouracil-treated male chicks at a normal weight were 0.000074 per cent of crystalline D,L-thyroxine in the feed, 0.000035 per cent of the monosodium salt, and 0.000038 per cent of the disodium salt. Comparative figures for the female chicks were 0.000078 per cent, 0.000036 per cent and 0.000039 per cent, respectively (table 1).



Figs. 1 and 2. METHOD OF PLOTTING DATA to determine the relative oral potency of D,L-thyroxine and its salts. The point at which the line representing the thyroids of thiouracil-treated chicks fed thyroxine crosses the line representing the thyroid weight of normal chicks indicates the amount of D,L-thyroxine in the feed necessary to maintain normal thyroid weight; i.e. to maintain a blood level of thyroxine equivalent to the amount of hormone normally produced.

As was anticipated, the results of this experiment indicate some relationship between the solubility of the compound administered and its physiological activity. However, the differences noted in the compounds studied are not as marked as might be expected. For example, the difference in solubility between monosodium and disodium thyroxine would lead one to expect some difference in the activity of these salts in favor of the disodium form. On the contrary, however, they seem to be practically equal in activity. In fact, our results indicate that the disodium salt seems to be slightly less active. However, we believe that this discrepancy is negligible and within the limits of experimental error.

Also, the magnitude of the differences between the activity of crystalline thyroxine and its salts appears somewhat less than might be expected. The results obtained show that the monosodium and disodium salts of thyroxine, when administered orally, are approximately twice as active as the pure crystalline form.

Although these observations do not show the exact amount of thyroxine absorbed, an approximation of this figure can be calculated. Chicks of the age used in this experiment consume, on the average, 20 to 25 grams of feed a day. The ration containing 0.000075 per cent of crystalline thyroxine would therefore supply about 15 gamma of thyroxine per chick per day. This amount is about five times that required when D,L-thyroxine is injected subcutaneously in alkaline solution (10). Therefore, assuming that the injected thyroxine is utilized 100 per cent, then approximately 20 per cent of the orally administered crystalline thyroxine is absorbed. On the same basis, 45 per cent of the sodium salts of thyroxine is absorbed.

DISCUSSION

Clearly our results are at variance with those of Thompson *et al.* (3), mentioned previously. There are several possible explanations for these differences. Species difference is an obvious possibility. Assay technique is another. The fact that these workers found a difference between the monosodium and disodium salts, whereas we did not, might be explained by their using thyroxine in alkaline solution as the disodium form in contrast to our use of a solid disodium salt. It must also be remembered that Thompson *et al.* observed only three patients and these had a definite myxedema. And, as those observers point out, the effect of thyroxine on the basal metabolism of such patients may be greater than in normal persons.

The work of Elmer and Rychlik (2) on urinary iodine excretion shows by inference that thyroxine administered orally in alkaline solution is absorbed a little more than twice as readily as pure crystalline thyroxine. This observation is in agreement with our results. It must be admitted, however, that the observations of Elmer and Rychlik may be somewhat misleading. Since no thyroxine can be detected *per se* in the urine (6, 11), the increment in urinary iodine caused by thyroxine administration must be due to some decomposition product of the hormone. And while it may be reasonable to assume that more thyroxine must be absorbed in order to promote this mode of iodine excretion, it is by no means the only excretion route for circulating thyroid hormone (12).

Schittenhelm and Eisler (4) arrived at a figure not too different from ours regarding the absorption of crystalline thyroxine. They found that 14 per cent of the crystalline thyroxine was absorbed, while our calculations indicate that about 20 per cent is absorbed. However, these investigators found 90 per cent of the thyroxine in alkaline solution is absorbed. There is a considerable discrepancy between this figure and our calculated 45 per cent for the solid disodium salt of thyroxine. Two possible explanations are suggested for this difference: 1) the thyroxine already dissolved in alkaline solution may be more readily absorbed than the solid disodium salt, and/or 2) as it is absorbed, more of the thyroxine may be inactivated and therefore would not manifest itself in our measurements. Also, Schittenhelm and Eisler introduced the alkaline solution of thyroxine directly into an isolated loop of intestine, whereas we fed the disodium salt. It is possible that not all of the thyroxine remained as the disodium salt, due to pH changes in the gastrointestinal tract. This might also be a partial explanation for our finding no difference between the activity of the monosodium and disodium salts of thyroxine.

Considering the overall picture, then, it appears that pure crystalline D,L-thyroxine is less effective when administered orally than are its more soluble sodium salts. Thyroxine already dissolved in alkaline solution may be even more effective. Apparently, therefore, the solubility of the compound administered is an important factor—as has been suggested by several investigators—but it appears not to be the only factor to be considered.

SUMMARY

An investigation was conducted to determine the relative oral effectiveness of crystalline, monosodium, and disodium D,L-thyroxine in the domestic fowl. It was found that 0.000074 per cent of crystalline D,L-thyroxine in the feed was the amount necessary to maintain the thyroids of thiouracil-treated male chicks at normal weight. Likewise, 0.000078 per cent was the amount required by the females. The requirements of the male chicks for the monosodium and disodium salts were found to be 0.000035 per cent and 0.000038 per cent, respectively. The comparative figures for the females were found to be 0.000036 per cent and 0.000039 per cent. It was calculated that crystalline thyroxine was absorbed to the extent of approximately 20 per cent, and the sodium salts, about 45 per cent.

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A STUDY OF LIPOTROPIC FACTORS DERIVED FROM THE PANCREAS¹

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SINCE MacLeod, in 1930, showed that feeding raw pancreas prevented the development of fatty livers in insulin-treated, depancreatized dogs (1, 2), interest has centered on the identity of the factor responsible for this action.

The interpretation of this problem has kept pace with subsequent investigations in the field of anti-fatty-liver substances and thus the original enzymatic theory of MacLeod (2) gave place to the lipotropic theory following the discovery of the lipotropic activity of lecithin (3, 4), choline and choline-like substances (5, 6), protein (6, 7), methionine (8) and inositol (9).

On the basis of that evidence choline has been looked upon as the ultimate mobilizing agent of the fat accumulated in the liver, the activity of the other agents being determined by their capacity to furnish choline on splitting (lecithin), to supply labile methyl groups for its synthesis (methionine and methionine-containing compounds, like proteins) or to replace choline in the formation of phospholipids (inositol and choline-like substances).

But when Dragstedt *et al.* (10, 11) and Entenman *et al.* (12) succeeded in obtaining pancreas extracts lipotropically active though practically choline-free, the existence of a new antifatty-liver factor present in pancreas was postulated by each group. Certainly, the interpretation offered by these two groups of workers was as different as the methods they used for the preparation of the pancreatic extracts. Dragstedt maintained that the fat-metabolizing agent present in his alcoholic extract was an internal secretion of the pancreas other than insulin, and named it lipocaic (11). He claims that its activity cannot be accounted for by its choline, protein, methionine or inositol content (10, 13).

On the other hand, Chaikoff and his group gave the name of antifatty-liver factor to the lipotropic principle contained in their choline-free pancreatic fraction C27. The action of this factor was first explained on enzymatic grounds postulating that it was able to release and thus make available for lipotropic purposes the bound methionine of food proteins (14). But with the recent preparation of purified pancreatic fractions highly lipotropic although devoid of proteolytic activity, Chaikoff and Entenman were led to concede that this hypothesis, at least in its simple form, might not be adequate (15).

The discrepancies between the two groups of investigators in spite of the fact that both assayed their preparations on the depancreatized or pancreatic duct-

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ligated dog, suggested to us the possibility that the lipotropic agent present in each extract could be different. Recently Chaikoff and Entenman stated, on the basis of their latest findings (15), that the pancreas might contain more than one unidentified antifatty-liver factor.

In view of the alleged efficacy of the oral and parenteral administration of lipocaic in preventing the hepatic fatty infiltration in rats fed a low-protein, high-fat diet (10), we thought that the comparative assay of both Dragstedt's and Chaikoff's extracts on that particular type of fatty liver would help to show whether or not the lipotropic agent present in them was the same.

Other experiments were conducted. One was designed to investigate the possibility that the activity of lipocaic is due to the combined action of the methionine and inositol present in it, since Dragstedt only proved that it could not be accounted for by its content of each of those substances separately (10, 13).

Oral and intravenous inositol was then tried on the pancreatic duct-ligated dog to see whether this compound displayed here its well known antifatty-liver activity studied by Gavin and McHenry in the rat (9). Finally the hypothetical lipotropic properties of trypsin, chymotrypsin and carboxypeptidase were investigated, on the basis of the fact, reported by Best (16), that Palmer found evidence of the presence of trypsin, chymotrypsin and other proteolytic enzymes in pancreatic fraction C27 of Chaikoff.

EXPERIMENTAL

Adult white rats weighing 150 to 250 gm. and mongrel dogs were used in our experiments. Fatty infiltration of the liver was produced in the rats by feeding them the low-protein, high-fat diet used by Dragstedt (11), and in the dogs by pancreatic separation performed as described by Montgomery, Entenman and Chaikoff (17). This technique gave us good results in a previous study (18). The dogs were fed a diet of raw meat, bread and milk. Blood samples were taken during fasting. The substances to be tested by mouth were given in gelatin capsules mixed with the food. No assay for lipotropic activity was carried out on the pancreatic duct-ligated dogs until at least 12 weeks had elapsed following the operation.

Lipocaic was obtained according to the last modification of the original technique of Clark *et al.* (10) and the lyophilized final product kept dry and cold. It contained 150 μ g. of choline per gram and 0.45 per cent methionine, as determined by microbiological methods (19).²

Fraction C27 was prepared following Entenman and Chaikoff's method (12). The final white dry powder was also stored in the cold. It contained no choline.

Both pancreatic preparations were readily soluble in water; the solutions for subcutaneous injection were made by dissolving the desired amount in the chosen volume of sterile saline, by aseptic technique.

Methionine. Eastman's product was used.

Inositol. The powdered product furnished by A. E. Staley Manufacturing Co. was used.

Trypsin and Chymotrypsin. Armour's crystalline preparations were used. They contain 50 per cent ammonium sulfate.

Carboxypeptidase. A pure preparation, kindly supplied by Dr. M. L. Anson, was used.

² Analysis conducted in laboratories of Armour and Company through the courtesy of Dr. J. H. Glynn.

Blood and Liver Analysis. Total phospholipids, total, free and esterified cholesterol, total fatty acids and alkaline phosphatase activity determinations were carried out on unhemolyzed dog serum. Liver total phospholipids, cholesterol and fatty acids were determined on aliquots of an alcohol:ether (3:1) extract of the organ. The following methods were used throughout the experiments:

Horecker, Ma and Haas' modification (20) of Fiske and Subbarow's (21) method for lipid phosphorus; Schoenheimer and Sperry's method (22) for total, free and esterified cholesterol with slight modifications (23); Marenzi and Cardini's method (24) for total fatty acids, (with prolongation of the saponification time); Shinowara, Jones and Reinhart's method (8) for alkaline phosphatase activity, adapting Holman's method (25) for the final measurement of phosphate, since it proved convenient in our hands (18).

All colorimetric determinations were done by means of a Coleman Spectrophotometer.

RESULTS

Comparative Lipotropic Activity of Two Pancreatic Extracts in Dietary Fatty Liver of the Rat. Four groups of 10 rats each (5 males and 5 females) were used in this experiment. Simultaneously with the feeding of the low-protein, high-fat diet the administration of the extracts was started. Subcutaneous injections were given to animals in groups 1 and 2 as follows: group 1, 167.0 mg. of lipocaic in 1 cc. of saline per day; group 2, 30.0 mg. of fraction C27 in 1 cc. of saline per day. Animals in group 4 received 30.0 mg. of fraction C27 per rat per day orally; group 3 was kept as a control.

After an experimental period of 20 days the animals were killed and the livers immediately removed, freed from blood as much as possible, weighed and then suspended in the alcohol:ether mixture by means of a Waring blender. After extraction under reflux the extracts were brought to a definite volume and filtered, aliquots of the filtrate being taken for determination of lipids. The results, given in table 1, show that only lipocaic was able to prevent the fatty infiltration of the liver in these rats.

As a control, the lipotropic activity of the pancreatic preparations used was tested on pancreatic duct-ligated dogs in which serum lipids had fallen below (26) and phosphatase activity risen above (18) pre-operative levels as a consequence of the fatty infiltration of the liver.

The restoration of serum lipids and phosphatase values to pre-operative levels after 20 (dog 260), 23 (dog 261) and 20 (dog 268) days' treatment, followed by a return of the low-lipids high-phosphatase pattern when the administration of the extracts was discontinued, was taken as proof of the lipotropic action of the preparations tested. Both pancreatic preparations were active, as shown in table 2.

Comparative Action of Parenteral Lipocaic, Methionine and Inositol on Dietary Fatty Liver of the Rat. Four groups of 5 rats each (males) were used in this experiment. The animals were kept on the lipogenic diet and injected daily according to the following schedule: group 2, 214.0 μ g of methionine in 1 cc. of saline per rat; group 3, 500.0 μ g of inositol in 1 cc. of saline per rat; group 4, 214.0 μ g of methionine plus 500.0 μ g of inositol in 1 cc. of saline per rat. Group 1 was kept as a control. The doses were calculated so as to give the amount of methionine and inositol present

TABLE 1. AVERAGE LIPID CONCENTRATION OF THE LIVER OF RATS FED A HIGH FAT, LOW PROTEIN DIET WITH AND WITHOUT SUPPLEMENTS (10 RATS IN EACH GROUP)

	PHOSPHOLIPID PHOSPHORUS	CHOLESTEROL			TOTAL FATTY ACIDS
		Total	Free	Ester	
	gm. %	gm. %			gm. %
Group 1 (lipocaic ¹)	4.3	0.147	0.121	0.016	3.88
Group 2 (fraction C27 ²)	4.5	0.214	0.140	0.074	15.40
Group 3 (control)	4.86	0.321	0.220	0.101	15.08
Group 4 (fraction C27 orally ³)	3.9	0.360			11.56

¹ 167 mg. of lipocaic subcutaneously daily.² 30 mg. of fraction C27 subcutaneously daily.³ 30 mg. of fraction C27 orally daily.

TABLE 2. EFFECT OF LIPOCAIC AND CHAIKOFF'S ANTI-FATTY-LIVER FACTOR UPON THE BLOOD LIPID LEVELS OF DOGS WITH LIGATED PANCREATIC DUCTS

	PHOSPHOLIPID PHOSPHORUS	CHOLESTEROL			TOTAL FATTY ACIDS	ALKALINE PHOSPHATASE
		Total	Free	Ester		
	mg. %	mg. %			mg. %	units %
Dog 260						
Before operation	15.8	175.5	52.6	122.9	420.0	7.0
Before oral Lipocaic	5.2	64.8	27.6	37.2	107.1	25.6
After 20 days' treatment	16.2	198.5	58.7	139.8	448.5	8.8
4 weeks after discontinuing treatment	6.0	79.2	31.7	47.5	114.0	28.8
Dog 261						
Before operation	17.0	156.8	43.1	113.7	460.0	4.6
Before oral C27 (100 mg.)	6.8	71.9	23.4	48.5	170.0	48.4
After 23 days' treatment	12.4	108.0	31.1	66.9	363.5	11.8
2 weeks after discontinuing treatment	10.0	88.2	25.8	62.4	220.0	28.6
Dog 268						
Before operation	17.4	186.0	55.8	130.2	412.5	5.0
Before oral C27 (100 mg.)	7.5	82.8	29.3	53.5	171.0	36.0
After 20 days' treatment	15.0	160.4	45.7	114.7	398.0	9.4
4 weeks after discontinuing	11.6	72.5	27.6	44.9	236.5	17.0

in 167.0 mg. of lipocaic—the amount proven effective in the preceding experiment—on the basis of the percentage figures given by Eilert *et al.* (13) and Abels (27).

After an experimental period of 24 days, the animals were killed and the livers immediately removed and analyzed as described. Results are given in table 3.

Comparing these values with those found for rats injected with lipocaic (*group 1* in table 1) it follows that the antifatty-liver activity of this pancreatic preparation can not be explained by its content of methionine or inositol.

Assay of the lipotropic activity of oral and parenteral inositol in the pancreatic duct-ligated dog. The ability of inositol to restore to pre-operative levels the lowered lipids and increased phosphatase activity of the serum of dogs with fatty livers induced by pancreatic separation, was tested in this experiment. Both the oral and the parenteral routes were tried according to the following plan:

Dog 249. 1.0 gm. of inositol per day orally, starting 20 weeks after the pancreatic separation. Treatment was continued for 28 days, discontinued for 1 week and re-started with the intravenous administration of inositol in daily doses of 1.5 gm. during 21 days.

Dog 268. 2.0 gm. of inositol per day orally, starting 24 weeks after operation. Treatment was continued for 28 days, discontinued for one week and re-started with the oral administration of 5 gm. of inositol per day during 20 days.

Dog 219. 1.0 gm. of inositol per day intravenously, starting 15 weeks after operation. Treatment lasted 28 days.

Dog 261. 1.0 gm. of inositol per day intravenously, starting 35 weeks after operation. The treatment lasted 28 days.

Dogs 261 and 268 had been previously used for the assay of fraction C27, and *dog 249* for trypsin, but enough time was allowed between tests in order to allow the animal to regain the characteristic blood picture known to accompany fatty infiltration of the liver.

Results are summarized in table 4. Inositol, either orally or subcutaneously, was unable to exert a marked lipotropic action in the pancreatic duct-ligated dog at the doses and duration of treatment used. At most a slight activity is suggested by the fact that it prevented a further drop of the serum lipids or rise of the hyperphosphatemia and in some cases induced a slight rise of the lipids.

Oral Administration of Crystalline Trypsin, Chymotrypsin and Carboxypeptidase to the Pancreatic Duct-ligated Dog. In order to determine whether trypsin or chymotrypsin could account for the activity of fraction C27, these enzymes were given to pancreatic duct-ligated dogs. Although, as far as we know, carboxypeptidase has not been reported to be present in fraction C27 this enzyme was also tried as a possible agent needed for the availability of ingested bound methionine for lipotropic purposes, according to Chaikoff's theory (14).

Unfortunately our supply of the crystalline enzymes was not sufficient for longer trials and in the particular case of carboxypeptidase we were restricted to a very short treatment (5 days). Notwithstanding, it is our impression that had this substance any lipotropic activity, it could have been demonstrated even in this short period as it was with fraction C27. The following doses were used:

Dog 268. Trypsin in daily doses of 100.0 mg. during 5 days, starting 13 weeks after pancreatic separation.

Dog 249. Trypsin in daily doses of 100.0 mg. during 5 days, starting 14 weeks after operation. Trypsin in daily doses of 100.0 mg. during 10 days, starting 37 weeks after operation.

Dog 249. Chymotrypsin in daily doses of 120.0 mg. during 12 days, starting 34 weeks after operation.

Dog 268. Chymotrypsin in daily doses of 10.0 mg. during 12 days, starting 37 weeks after operation.

Dog 249. Carboxypeptidase in daily doses of 100 mg. during 5 days, starting 25 weeks after operation.

TABLE 3. AVERAGE LIPID CONTENT OF THE LIVERS OF RATS ON A LIPOGENIC DIET RECEIVING INOSITOL, METHIONINE AND THE TWO SUBSTANCES TOGETHER IN THE AMOUNTS WHICH WOULD BE CONTAINED IN A THERAPEUTIC DOSE OF LIPOCAIC

	PHOSPHOLIPID PHOSPHORUS	TOTAL CHOLESTEROL	TOTAL FATTY ACIDS
	gm. %	gm. %	gm. %
Group 1 (control)	3.49	285.6	14.71
Group 2 (inositol ¹)	3.51	247.4	12.09
Group 3 (methionine ²)	3.45	254.6	9.03
Group 4 (methionine+inositol ³)	4.62	291.1	10.70

¹ 214 μ g. of methionine subcutaneously daily. ² 590 μ g. of inositol subcutaneously daily.

³ 590 μ g. of inositol plus 214 μ g. of methionine subcutaneously daily.

TABLE 4. BLOOD LIPID CONTENT OF PANCREATIC DUCT-LIGATED DOGS RECEIVING INOSITOL ORALLY OR INTRAVENOUSLY

	PHOSPHO- LIPID PHOSPHORUS	CHOLESTEROL			TOTAL FATTY ACIDS	ALKALINE PHOSPHATASE
		Total	Free	Ester		
	mg. %	mg. %			mg. %	units %
<i>Dog 249</i>						
Before operation	16.2	198.0	54.5	143.5	427.8	3.6
Before oral inositol (1 gm.)	4.6	46.2	18.5	27.7	99.0	40.8
After 28 days' treatment	5.0	18.6	8.1	10.5	101.0	49.4
Before I.V. inositol (1.5 gm.)	2.8	17.4	9.1	8.3		65.6
After 21 days' treatment	7.6	60.5	26.0	34.5	158.4	46.8
<i>Dog 268</i>						
Before operation	17.4	186.0	55.8	130.2	412.5	5.0
Before oral inositol (2 gm.)	11.6	72.5	27.6	44.9	236.5	17.0
After 28 days' treatment	12.0	78.0	31.5	46.5	250.0	15.8
Before oral inositol (5 gm.)	10.0	70.7	28.0	42.7	210.0	18.5
After 20 days' treatment	11.2	84.6	31.6	53.0	230.0	17.0
<i>Dog 219</i>						
Before operation	13.6	136.8	41.0	95.8	411.0	4.4
Before I.V. inositol (1 gm.)	5.8	73.4	29.4	44.0	171.8	17.6
After 28 days' treatment	5.8	57.0	22.8	34.2	119.2	18.0
<i>Dog 261</i>						
Before operation	17.0	156.8	43.1	113.7	460.0	4.6
Before I.V. inositol (1 gm.)	8.0	74.7	27.6	47.1	179.9	13.0
After 28 days' treatment	8.6	55.2	17.7	37.5	172.6	19.6

As in the preceding experiments some of the dogs had been used for testing other substances, or the same substance at varying intervals of time, but in every case the administration of the product to be assayed was not started until blood values had reached abnormal levels, as shown in table 5.

The results in table 5 show that none of the enzymes was able to restore to normal the serum lipids and phosphatase activity of pancreatic duct-ligated dogs, although some trend in that direction was shown by dog 268 when given trypsin.

TABLE 5. BLOOD LIPID VALUES OF DOGS WITH LIGATED PANCREATIC DUCTS BEFORE AND AFTER FEEDING CRYSTALLINE TRYPSIN, CHYMOTRYPSIN OR CARBOXYPEPTIDASE

	PHOSPHO- LIPID PHOSPHORUS	CHOLESTEROL			TOTAL FATTY ACIDS	ALKALINE PHOSPHA- TASE
		Total	Free	Ester		
	mg. %	mg. %			mg. %	units %
<i>Dog 268</i>						
Before operation	17.4	186.0	55.8	130.2	412.5	5.0
Before oral trypsin (100 mg.)	7.5	82.5	33.0	49.5	162.0	28.6
After 5 days' treatment	12.4	105.7	35.4	70.3	300.0	15.6
Before oral chymotrypsin (120 mg.)	9.4	57.0	21.0	36.0	195.5	15.8
After 12 days' treatment	9.8	63.5	24.4	39.1	203.8	19.0
<i>Dog 249</i>						
Before operation	16.2	198.0	54.5	143.5	427.8	3.6
Before oral trypsin (100 mg.)	6.0	42.0	16.0	26.0	125.0	42.4
After 5 days' treatment	6.4	54.6	18.8	35.8	145.5	26.8
Before oral trypsin (100 mg.)	4.6	35.4	15.6	19.8	98.6	43.0
After 10 days' treatment	4.6	28.2	13.5	17.7	97.4	20.6
Before oral chymotrypsin (120 mg.)	4.6	37.7	13.6	24.1	97.0	38.2
After 12 days' treatment	4.6	35.4	13.8	21.6	98.6	32.4
Before oral carboxypeptidase (100 mg.)	5.0	18.6	8.1	10.5	101.0	49.4
After 5 days' treatment	2.8	17.4	8.1	8.3		65.6

DISCUSSION

The fact that Dragstedt's lipocaic and Chaikoff's pancreatic fraction C27 were both lipotropically active by mouth in the pancreatic duct-ligated dog, while only the former was able to prevent the fatty infiltration of the liver in the rat when administered parenterally seems to prove that the lipotropic principles responsible for the activity of those extracts are not the same substance.

The possibility of the anti-fatty-liver properties of lipocaic being due to choline is not likely in view of the fact that the dose used (167 mg.) contained only 25 μ g. of choline. Furthermore, *experiment 2* rules out methionine and inositol, either separately or together, as the main lipotropic factors of the extract. This confirms previous work by Clark *et al.* (10) and by Owens *et al.* (28) and supports the statement that the lipotropic principle of the pancreas present in lipocaic is a specific substance still unidentified. It remains to be proved whether or not it is of a hormonal nature.

As Chaikoff and Entenman (15) point out—and we agree with them—the type of fatty liver induced in the rat by a low-protein, low-choline, high-fat diet, should

not be used as a specific test object for the pancreatic anti-fatty-liver factor. However its employment for comparative purposes in the present study has demonstrated an interesting difference in lipotropic substances.

The negative response obtained with inositol in the dog both orally and parenterally, offers a further proof that this is not the specific lipotropic factor of lipocaic. On the other hand, Abel *et al.* (27) reported that the lipotropic properties of lipocaic, as tested in patients, could be accounted for by its content of inositol.

The inability of trypsin, chymotrypsin and carboxypeptidase to reproduce the effect of the same amount of fraction C27 in the dog seems to be against the assumption that Chaikoff's anti-fatty-liver factor is of a proteolytic nature, and is more in accordance with recent findings by Chaikoff and Entenman (15).

SUMMARY

Extracts of pancreas prepared according to Chaikoff's or Dragstedt's method when administered orally to dogs raise the level of blood lipids which has been caused to fall by ligation of the pancreatic ducts. Dragstedt's extract (lipocaic) also prevents the fatty liver which occurs when rats are placed upon a high-fat, low-protein diet; Chaikoff's extract does not. Inositol, choline, or the two substances together have no influence upon the dietary fatty liver of the rat when given in amounts equivalent to those found in lipocaic. Inositol orally or parenterally and crystalline trypsin, chymotrypsin and carboxypeptidase by mouth are not lipotropic in the pancreatic duct-ligated dog.

Two separate, as yet unidentified factors, Dragstedt's lipocaic and Chaikoff's antifatty-liver factor, which are extractable from hog pancreas, are capable of exerting lipotropic activity in the dog with ligated pancreatic ducts. Inositol, choline, methionine, trypsin, chymotrypsin and carboxypeptidase fail to account for the lipotropic action of either of these factors.

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URINARY AND GENITAL TRACT PHOSPHATASES OF THE MALE DUTCH RABBIT¹

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THE discovery of large amounts of an acid phosphatase in the human prostate by Kutscher and Wolbergs (1) has led to considerable interest in genital tract phosphatases, although most of the work has been confined to man. The occurrence of human prostatic acid phosphatase has been demonstrated in prostatic tissue and secretion and in seminal fluid, as well as in blood serum and urine, by various workers (e.g., 2-8).

Much of the significance attached to clinical determinations of acid phosphatase activity of serum and urine is due to the striking increase in levels that accompany most metastasizing prostatic carcinomas, where the measurement of prostatic acid phosphatase is considered a valuable diagnostic aid (9, 10). The possible function of this phosphatase has been discussed by Sullivan, Gutman and Gutman (8) and investigated by Lundquist (11, 12), who found that phosphorylcholine was the natural substrate for the acid phosphatase in human seminal fluid.

A similar acid phosphatase in amounts appreciably larger than in other tissues has been reported in rat preputial gland (13), in dog prostate (13-15), and in the caudal lobes of the monkey prostate (16). High alkaline phosphatase activity has been reported for the rat prostate and preputial gland (13) and the monkey prostate (16). The alkaline phosphatase in the rat prostate has since been shown to be confined largely to the ventral lobes (17). Atkinson (18) has recently demonstrated histochemically strong alkaline phosphatase activity in the stroma of the seminal vesicle of the mouse which disappears with castration.

Gutman and Gutman (13) reported low acid phosphatase activity in rabbit prostate, similar to that found in most non-genital tissues. The present study was undertaken to investigate more thoroughly the occurrence of genital tract phosphatases in the male rabbit and their possible manifestation in urine.

MATERIALS AND METHODS

Animals

Thirty-nine adult male rabbits, some with experimentally induced endocrinopathies, were used at various times in these experiments as a source of urine. Six of these and 4 others were dissected for tissue assays, and 4 provided feces. All except 2 rabbits were Dutch stock, the 2 exceptions being Dutch hybrids. All animals received a diet of standard prepared pellets, supplemented by carrots and/or cabbage once or twice a week. There was no temperature control.

Preparation of Materials for Testing

Urine. Urine was collected either directly from the bladder post mortem or expressed through the urethra with the animal under nembutal anesthesia or post

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mortem. A catheter was not used since it would prevent the addition of any sex accessory secretions normally entering the urine. Varying volumes, from 1 to 30 ml., were obtained. These were centrifuged for 10 to 15 minutes to eliminate the heavy sediment present in rabbit urine. The sediment was not tested for the phosphatase activity which might have been present, particularly if cellular elements were centrifuged down with the salts (19). Thus, the 'urine' referred to herein actually represents the supernatant after centrifugation. Centrifuged rabbit urine is dark amber in color.

Tissue. Certain tissues were tested semi-quantitatively for the occurrence of the phosphatases. Fresh weighed tissue (distal end of the seminal vesicle, prostate-vesicular gland, bulbo-urethral including striated muscle, testis-epididymis) was placed in a Waring blender with a known volume of distilled water (50-70 ml. for the large blender bowl) and macerated for 5 minutes with this apparatus. The resultant mixture was centrifuged for 20 minutes, and the supernatant tested for phosphatase activity. It is important to point out that complete homogenation was not carried out, and that determinations on the extracts give an indication of the relative phosphatase activities in the several accessories tested, and not the absolute amounts. Moog and Steinbach (19) have demonstrated that some tissue phosphatase is absent from extracts (bound in cytoplasmic granules), and that total tissue homogenates are desirable for complete assays.

Feces and Food. To determine the occurrence of phosphatases in commercially prepared food pellets and in feces, 5 gm. of each sample were homogenized in 50 ml. of distilled water in the Waring blender for 10 seconds. The food mixture was then filtered for one hour; the feces for 20 minutes. The filtrates were then centrifuged for 15 minutes, and the supernatants tested as described above. The final fecal extracts were cloudy; the final food extracts turned bright green when an alkaline pH was attained.

Phosphatase Determination Technic

There are several methods available for phosphatase determination, involving the enzymatic hydrolysis of organic phosphates. The method employed herein is that of Huggins and Talalay (20), utilizing the breakdown of sodium phenolphthalein phosphate and the colorimetric determination of the free phenolphthalein. Inasmuch as the relationship between the phenolphthalein produced and the enzyme concentration is parabolic (probably due to the existence of two hydrolytic products of phenolphthalein diphosphate), it is necessary to express enzyme activity in arbitrary units instead of mg. of phenolphthalein liberated. Huggins and Talalay claim that the method can be employed in human urines without the necessity of dialysis to eliminate chromogens.

The test as modified was conducted as follows: The urine (or other test solution) was incubated at 37° C. in a water bath with the appropriate acid or alkaline buffered substrate for one hour. At the end of this time the reaction was terminated by the addition of glycine buffer (pH 11.2) containing pyrophosphate as an enzyme inhibitor and the maximum color of phenolphthalein obtained (attained at pH 10.5-11.4). One ml. of urine was used with 10 ml. of the particular substrate (in acetate buffer,

pH 5.4, for the acid phosphatase; in barbiturate buffer, pH 9.7, for the alkaline phosphatase). Nine ml. of glycine buffer were used to terminate the reaction, making a final total volume of 20 ml. The color was read within a few minutes after termination in an Evelyn photoelectric colorimeter using a 540 filter. (Within 15 minutes after termination, the color was found not to alter appreciably.) The tubes were equilibrated in a 37° C. water bath for about 5 minutes before the urine was added to the substrate. Determinations were made in duplicate or triplicate, except for single determinations in a few cases when the amount of urine was insufficient and for occasional determinations in quadruplicate.

The blank employed consisted of the total reaction mixture of substrate plus inhibiting glycine buffer plus test solution, incubated along with the test solution-substrate mixtures. This blank was chosen to correct for any slight turbidity and for the definite color of the urine, as well as for any free phenolphthalein in the substrate. The recommended blank (20) is distilled water plus buffer plus test solution and does not correct for free phenolphthalein in the substrate.

Some urines tested for acid phosphatase produced a slight negative deflection of the galvanometer, as compared with the acid phosphatase blank. The reason for this is not known, but it may be due to the effect of the alkaline pH on the urinary chromogens after hour-long exposure in the blank tube, or to the effect of the acid pH in the acid buffered test tube. Thus, our blank, like that of Huggins and Talalay, is not a complete control, and some error may be introduced by the pH effect on the urinary chromogens of the blank as compared with that of the substrate. The negative deflections are reported as 0 units of acid phosphatase in our tables.

It was found that the original acid and alkaline buffered substrate solutions contained appreciable amounts of free phenolphthalein after dissolving the crystalline salt (purchased from Paul-Lewis Laboratories, Milwaukee, Wis.). Approximately 200 ml. portions of each stock substrate solution were extracted with five successive 20-ml. portions of ethyl ether in a separatory funnel and then aerated to remove traces of ether. The color reading for the unextracted buffers indicated the presence of less than 10 mg. of free phenolphthalein per liter, resulting from the decomposition of approximately 20 mg. of the salt. Inasmuch as Huggins and Talalay state that "the rate of hydrolysis is independent of substrate concentration between 0.0005 M and 0.002 M," the 20 mg. per liter decrease in the substrate molality of 0.001 M (0.608 gm/l.) should not affect the reaction rate. A straight line was obtained when known concentrations of phenolphthalein were plotted against the color density. This provided a standard phenolphthalein calibration curve for conversion of the color density values into mg. of phenolphthalein.

Dilutions of solutions containing acid and/or alkaline phosphatase were prepared, and curves were constructed by plotting mg. of phenolphthalein liberated per 100 ml. of test solution against concentration. This relationship is parabolic, and hence values were plotted on log-log graph paper to obtain a straight line. We used Huggins and Talalay's definition of units of activity: "10 units is the amount of enzyme which will liberate the colorimetric equivalent of 1 mg. of phenolphthalein from excess substrate in 1 hour at 37° C. under optimum conditions of pH ." The mg.-unit curve is a line drawn parallel to the curves obtained above through the reference point

of 10 units = 1 mg. The curve shown in figure 1 represents the results of diluting five acid and alkaline phosphatase preparations (two rabbit testis-epididymis extracts and a diluted rabbit urine containing spermatic fluid). The slope of this average curve is slightly less than of the curve obtained by Stafford, McShan and Meyer (21) using rat pregnancy corpora lutea homogenates, and both are less steep than Huggins and Talalay's original curve based on serum phosphatases.

Although several determinations were made in triplicate and quadruplicate, most were made in duplicate. The variations in all cases were small. To indicate the reliability of the technic, a correlation coefficient (r_{xy}) of 0.996 was obtained for

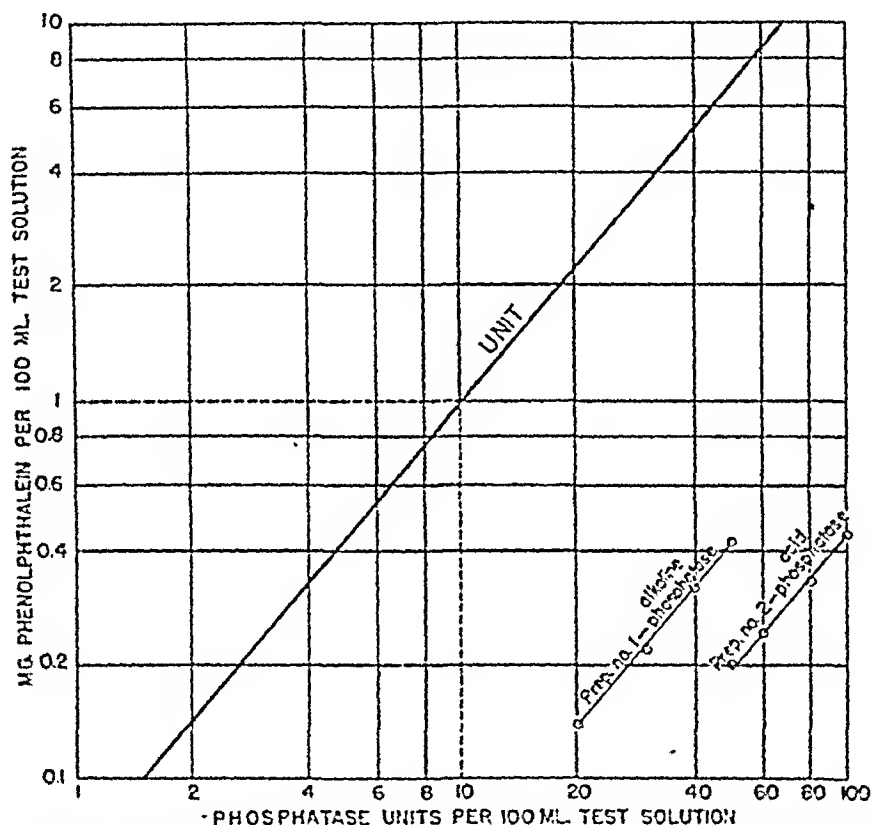


Fig. 1. LOG-LOG PLOT OF PHOSPHATASE UNIT CURVE. Two curves relating phosphatase concentration and mg. of phenolphthalein liberated are shown in lower right corner. Unit curve is line parallel to these through point: 10 units = 1 mg.

the first 36 pairs of determinations in the range 0 to 15. Thus, the large differences in samples from different animals are due to biologic variability and not to technical inadequacy. The results listed in all the tables are the means of the several determinations.

OBSERVATIONS AND DISCUSSION

Tables 1 to 3 indicate the range of values obtained for urinary acid and alkaline phosphatases in rabbits under various experimental conditions. These values are all based on centrifuged expressed urines. Among intact untreated animals (table 1), acid phosphatase varies from 0 to 10.5 U/100 ml. of urine, and alkaline phosphatase from 4.9 to 82 units. Table 4 lists several values for bladder urines, indicating the

presence of some acid and alkaline phosphatase in urine which has not contacted the urethral canal. The kidney is known to be rich in alkaline phosphatase (22, 23) and is a possible source of this enzyme in bladder urine. Alkaline phosphatase levels are evidently considerably higher in male rabbit urine than in male human urine.

TABLE 1. INTACT CONTROL RABBITS—EXPRESSED URINE PHOSPHATASES

ANIMAL	DILUTION PRIOR TO TESTING	UNITS PHOSPHATASE/100 ML.	
		Acid	Alkaline
1		3.6	13.5
2		0	16
3		0	6.9
4		0	4.9
5		0	6.9
6			50
7		0	7.4
8		0	5.1
9		0	6.0
10	0.5		9.1
11	0.5	0	11
12		0	44
13 ¹		0	22
	0.5 (alk.)	0	82
		0	18
14			13
15			22
16 ²	0.2	10.5	51

¹ Determinations on three consecutive days. ² Excretion of spermatic fluid noted.

TABLE 2. ANDROGEN-TREATED INTACT RABBITS WITH INTRAPROSTATIC METHYLCHOLANTHRENE PELLETS, 251-321 DAYS—EXPRESSED URINE PHOSPHATASES

ANIMAL	DILUTION PRIOR TO TESTING	UNITS PHOSPHATASE/100 ML.	
		Acid	Alkaline
CA-11	0.3		7.0
CA-12		0	
	0.2		79
CA-13 ¹		2.4	140
CA-14		0	13
CA-15		0	6.0

¹ Excretion of seminal vesicle fluid noted.

It was noted that whenever some seminal vesicle secretion or spermatic fluid was ejected with the expressed urine, values for alkaline phosphatase were very high (e.g., animal 16 in table 1, CA-13 in table 2). Thus, it was supposed that one or more of the accessories, the duct system and/or the testis produced alkaline phosphatase in appreciable amounts. To examine this possibility, extracts of the several

TABLE 3. ESTROGEN-TREATED RABBITS—EXPRESSED URINE PHOSPHATASES

ANIMAL	DILUTION PRIOR TO TESTING	UNITS PHOSPHATASE/100 ML.	
		Acid	Alkaline
GROUP A—Castrate rabbits receiving 10-40 injections of 0.02 mg. estrone daily			
LE-11A		3.6	25
LE-22A		0	5.3
	0.3		23
LE-33A	0.5 (alk.)	0	7.6
GROUP B—Castrate rabbits with estrogen pellets implanted, 433-633 days			
EST-1	0.2		20
EST-2		0	2.0
		0	2.6
	0.5		2.9
EST-8		0	4.6
	0.5 (alk.)	0	9.6
	0.5	0	6.6
GROUP C—Castrate rabbits with estrogen pellets and intraprostatic methylcholanthrene pellets implanted, 297-372 days			
CA-16 ¹	0.1	0	30
CA-18			8.6
GROUP D—Intact rabbits with estrogen pellets implanted, 442-582 days			
EST-4		0	8.2
EST-7	0.5	0	5.5
	0.5 (alk.)	0	4.1

¹ Urine mostly mucus.

TABLE 4. INTACT CONTROL RABBITS—BLADDER URINE PHOSPHATASES

ANIMAL	DILUTION PRIOR TO TESTING	UNITS PHOSPHATASE/100 ML.	
		Acid	Alkaline
8	0.5	0	14
10			11.3
11	0.5		12
12		0	5.0
17	0.2	0	9.5
18	0.2	0	23
19		4.6	18

accessories and of the testis-epididymis were prepared and tested for acid and alkaline phosphatase activity (table 5).

It can be seen that the secretion-containing seminal vesicle and the testis-epididymis are rich in alkaline phosphatase as compared with the other organs tested.

They represent possible sources of high alkaline phosphatase activity in urine. The absence of measurable alkaline phosphatase in seminal vesicles not containing secretion may indicate that only the active seminal vesicle is phosphatase-producing or that such activity is derived from admixture of spermatic fluid from the vasa (which empty into the seminal vesicle duct in the adult rabbit) with the normal secretion. In addition, the occurrence of acid and alkaline phosphatase in tissue extracts does not prove that these enzymes are present in the secretions of the glands. It is surprising that acid phosphatase values in urine are generally low, even when alkaline phosphatase values are high, especially since all the organs tested generally show appreciable acid phosphatase activity. The only exception (*animal 16* in table 1), with 10.5 U/100 ml., occurred in a urine which was diluted 1:4 prior to testing.

The data in table 5 indicate higher acid phosphatase activity in the prostate-vesicular gland than would be expected judging from Gutman and Gutman's (13)

TABLE 5. INTACT RABBITS—TISSUE EXTRACT PHOSPHATASES

ANIMAL	UNITS PHOSPHATASE/GM. OF FRESH TISSUE							
	Bulbo-urethral		Seminal Vesicle		Prostate-Vesicular Gland		Testis-Epididymis	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
7							21	25
14	4.7	0	15	15	19	0	47	38
17	9.5	0	14	23	13	0		
18	15	0	4.8	13	17	0		
20	3.8	0	15	0 ¹	14	0	23	24
21	11	0	0	0 ¹	36	1.7	50	55
22 ²	0	0	0	0 ¹	38	1.5	47	43
23 & 24			12	21				
25 ²			3.0	11	8.2	0		

¹ Devoid of secretion. ² Testosterone pellet implanted subcutaneously 10 days previously.

observations on the rabbit using the King-Armstrong technic (1.9 King-Armstrong U/gm. of fresh tissue).

Due to the wide variations in both normal and experimental values for urinary acid and alkaline phosphatase, it is not considered justifiable to draw any conclusions from these studies as to the effect of steroids and/or methylcholanthrene on urinary phosphatases. The absence of seminal vesicle or spermatic fluids is the probable reason why those animals receiving estrogen (table 3) did not show the extremely high alkaline phosphatase values found in some normal (50–82 U/100 ml.) and in some androgenized animals (79 and 140 U/100 ml.) (tables 1 and 2).

One of the difficulties encountered in this study was the small volumes of urine available. In an attempt to overcome this, urine collection cages were set up on several rabbits. In urine so collected, the acid phosphatase values were often quite high (up to 84 U/100 ml.). Inasmuch as it was impossible to avoid contamination of the urine with an occasional small fecal pellet or food fragment (even using very fine mesh screen could not eliminate the possibility of the animal's urinating over its own fecal deposits), it was considered that this material might account for the high acid phosphatase activity.

Feces were found to contain 18 to 30 units of alkaline phosphatase and 4.0 to 8.2 units of acid phosphatase³ per gram. Prepared food pellets, on the other hand, showed very high acid phosphatase activity (47-128 U/gm.) and virtually no alkaline phosphatase (0.5-0.6 U/gm.), and are a possible source of acid phosphatase not only in the collected urine, but in feces. The alkaline phosphatase in the feces may originate in the intestinal mucosa (22, 23). In addition, some acid and alkaline phosphatase could be produced by the bacteria in feces and by bacterial growths in standing urine. The inability to insure absence of incidental contamination of urine with feces and food, then, made collection cages, as customarily employed for urinary steroid determinations, useless for our purpose. It was necessary to dilute small volumes of expressed and bladder urines (0.1-0.5) with distilled water to obtain duplicate or triplicate runs.

It was noted when preparing dilution curves based on urines collected for 24 or more hours, under conditions described above, that the results of dilution were erratic. This was particularly true for the acid, but also to a slight extent for the alkaline, phosphatase. Whereas the alkaline phosphatase activities, although consistently higher, corresponded approximately to the expected values, the acid phosphatase activities increased with dilution in one animal and were consistently above the expected values in the others.

This phenomenon may indicate the presence of an inhibitor or inhibitors of acid phosphatase activity, at least in urine collected under conditions allowing some contamination with feces and bacteria. Robinson and Warren (24) have recently concluded that human urine shows similar acid phosphatase inhibitor activity. In this connection a statement by Gutman and Gutman (13) on human prostate and rat preputial gland tissue acid phosphatase is of interest: "Further dilution of the extract as high as 1:500 was necessary for optimal hydrolysis." However, we did not note inhibition in preparing the curves from testis-epididymis extract dilutions as described above.

If feces contain inhibitors to acid phosphatase activity, the low acid phosphatase values obtained for feces (on aqueous extract dilutions of 0.1 gm. and 0.01 gm/ml. solution) may be meaningless. In addition, if an inhibitor be present in uncontaminated urine, the negative values obtained in most urines for acid phosphatase activity would be understandable. The presence of acid phosphatase in the several accessories and the large amounts in the testis-epididymis should be evidenced in the urine as measurable acid phosphatase activity.

Because of the possibility of contact of urine with feces and/or food pellets which contain large amounts of enzymes and because of the presence of possible inhibitors, it would seem that urinary phosphatase level determinations in the male rabbit are of dubious value in the study of experimentally induced sex accessory pathologies.

SUMMARY

Alkaline and acid phosphatase activity of genital tract tissue extracts and of urine collected under several conditions from male Dutch rabbits was determined

³ For reasons discussed below (presence of inhibitors), the relatively low acid phosphatase values may not be a real indication of enzyme activity in feces.

using the Huggins and Talalay technic. There is a wide range of values for the appreciable amount of alkaline phosphatase found in male rabbit urine. Probably due to the decrease in normal seminal secretions, estrogen-treated rabbits do not show high values (above 30 units/100 ml. urine). As a rule, there is no appreciable acid phosphatase activity in uncontaminated urine. All accessories studied, as well as the testis-epididymis, and especially the prostate-vesicular gland, contain some acid phosphatase. Alkaline phosphatase is present in large amounts in the testis-epididymis, and generally in the seminal vesicle.

Urine collected under conditions allowing contamination with food or feces does not provide a reliable picture of phosphatase activities.

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VASCULAR REFRACTORINESS PRODUCED BY BENADRYL AND BAL

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SEVERAL YEARS ago we pointed out that one of the characteristic changes during terminal shock was the failure of blood vessels to respond when stimulated chemically (1, 2). This refractory state, which included the myocardium, was believed to be an important part of the mechanism of circulatory failure.

In an effort to produce vascular refractoriness experimentally without the associated changes incident to shock, such as hypovolemia, such substances as the antihistamine agents were employed. Although the action of Benadryl (β -dimethylaminoethyl benzhydryl ether-hydrochloride) is chiefly to annul the depressor action of histamine (this problem is ably reviewed by Loew, 3) Page and Green (4) presented some evidence that with large doses the pressor responses to adrenalin, tyramine, angiotonin and barium chloride were also significantly reduced. Further, mixing these agents with Benadryl and adding them to phosphate-containing Ringer's solution or citrated plasma perfusing through the vessels of isolated rabbit ears, greatly diminishes the vasoconstrictor response. The inhibition observed in intact animals must therefore have occurred chiefly at the smooth muscle fiber.

Augmentation rather than inhibition of the adrenalin response may occur with smaller doses of Benadryl. Loew, MacMillan and Kaiser (5) state: "Adequate doses of Benadryl augmented the pressor response to epinephrine. There was no evidence of epinephrine reversal which has been demonstrated with several other synthetic drugs which possess some degree of antihistamine action." The difference in their results and ours probably depends on the higher doses we used.

Since inhibition by Benadryl to the vasoactive test drugs was never complete, a search was made for some substances which would complete it. After many trials, BAL was finally chosen. BAL acts chiefly by binding the essential thiol groups in enzyme proteins. Thus, it was not unexpected that enzyme systems unaffected by Benadryl were altered by BAL.

The simultaneous administration of these substances prevents the pressor or depressor and direct vascular actions of a variety of vasoactive substances. These changes were studied concurrently in intact animals and in the isolated rabbit-ear vessels. Despite vascular refractoriness, arterial blood pressure may be maintained at normal levels for as long as an hour. The sudden fatal collapse which sooner or later develops has so far been irremediable.

It also seemed of interest to determine the ability of the refractory vascular tree to withstand increased pressure and volume of blood. For this purpose, pressure

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and volume were altered experimentally by means of arterial transfusion. But of chief importance to us seems to be the artificial reproduction of vascular refractoriness to chemical stimuli and maintenance of arterial pressure followed by vascular collapse somewhat as it occurs in shock.

METHODS

Mongrel dogs were anesthetized by intraperitoneal injection of 30 mg/kg. of sodium pentobarbital. The femoral artery was cannulated for blood pressure registration and all injections were made into the femoral vein. Benadryl was usually given in doses of 100 mg. either intravenously or intramuscularly or both, until the depressor response of histamine was almost abolished. BAL was given as an emulsion intraperitoneally, intravenously or intramuscularly. The stimulating substances were employed in the following dosages: 0.2 cc. adrenalin 1:10,000; choline, 16 mg.; histamine, 0.04 mg.; barium chloride, 9 mg.; angiotonin, 8 units; tyramine, 1.0 mg.

The isolated rabbit's ear was perfused by the method described by Page and Green (6). Benadryl solution (0.2 cc. containing 0.02 mg.) was added to various vasoconstrictors and injected into the stream perfusing the ear vessels. Control injections without Benadryl were made also. The results were recorded in terms of the number of minutes, the drop rate was reduced below the control value, and the percentage reduction as well.

The effect of increasing intravascular pressure in the experimental animals was measured by a reservoir recording on kymograph paper. The pressure within the reservoir was controllable. A cannula in the femoral artery was connected by plastic tubing to the reservoir. This apparatus has been described by Glasser and Page (7). It provides a satisfactory way of measuring the uptake and output of blood or other fluid under a controllable arterial pressure.

RESULTS

Intact Animals

Benadryl and BAL Treatment. Repeated intravenous injection of Benadryl in 100-mg. doses causes the adrenalin response to decrease to about 60 per cent of the control value, but it usually does not go much below this (table 1). Histamine responses, on the other hand, are more quickly and more completely abolished. The responses to angiotonin and barium chloride are reduced but not abolished.

BAL alone in doses of 0.5 cc. intravenously or 2 cc. intramuscularly abolishes the adrenalin response in some experiments, while arterial pressure is only moderately reduced. In most, the reduction averages almost 70 per cent of the normal response. The effect on histamine is insignificant. The response of tyramine appears to be reduced, but it is difficult to be certain because of tachyphylaxis. BAL also reduces slightly the depression of arterial pressure by choline. Angiotonin and renin responses are not conspicuously affected.

When the two drugs are combined, usually by giving repeated doses of Benadryl by vein and BAL intraperitoneally and intramuscularly, the responses to all of these pressor and depressor substances may be reduced significantly or abolished. Most importantly (table 2) the arterial pressure is often maintained at normal levels for periods ranging from 10 minutes to an hour. But sudden fatal collapse may occur without warning at any time. None of these animals survived, even though attempts were made to maintain pressure by intra-arterial transfusion with blood or normal salt solution.

Effect of Benadryl on Isolated Blood Vessels

Since Benadryl reduces the responsiveness of the vascular tree in intact animals, it seemed of interest to ascertain whether the same phenomenon would be demon-

TABLE 1. EXAMPLE OF EFFECT OF BENADRYL ON VASCULAR RESPONSIVENESS

SUBSTANCE INJECTED	INITIAL D.P.	CHANGE IN D.P.	TIME	SUBSTANCE INJECTED	INITIAL D.P.	CHANGE IN D.P.	TIME
	mm. Hg	mm. Hg			mm. Hg	mm. Hg	
Adrenalin 0.2 cc.....	144	82	10:30	Adrenalin 0.2 cc.....	80	28	11:55
Adrenalin 0.2 cc.....	144	90	10:34	Histamine 0.04 mg.....	76	-8	11:59
Histamine 0.04 mg.....	136	-44	10:37	Adrenalin 0.2 cc.....	82	38	12:38
Angiotonin 8 units.....	140	40	10:44	Adrenalin 0.2 cc.....	84	42	12:59
Benadryl 0.1 gm.....			10:55	Benadryl 0.1 gm.....			1:04
Adrenalin 0.2 cc.....	122	32	10:59	Angiotonin 8 units.....	76	14	1:12
Benadryl 0.1 gm.....			11:00	Adrenalin 0.2 cc.....	72	38	1:16
Adrenalin 0.2 cc.....	118	44	11:02	Histamine 0.04 mg.....	70	-8	1:20
Benadryl 0.1 gm.....			11:07	Adrenalin 0.2 cc.....	80	42	2:00
Adrenalin 0.2 cc.....	104	36	11:14	Adrenalin 0.2 cc.....	80	50	2:20
Benadryl 0.1 gm.....			11:20	Barium chloride 9 mg...	84	36	3:02
Adrenalin 0.2 cc.....	64	34	11:22	Benadryl 0.1 gm.....			3:06
Adrenalin 0.2 cc.....	96	40	11:42	Barium chloride 9 mg...	90	18	3:10
Benadryl 0.1 gm.....			11:47	Dog weight 15 kg.....			

TABLE 2. EXAMPLE OF COMBINED EFFECTS OF BAL AND BENADRYL ON PRESSOR-DEPRESSOR RESPONSES OF AN ANESTHETIZED DOG

	INITIAL PRES- SURE	RISE OR FALL IN ARTERIAL PRESSURE	TIME		INITIAL PRES- SURE	RISE OR FALL IN ARTERIAL PRESSURE	TIME
	mm. Hg	mm. Hg			mm. Hg	mm. Hg	
Adrenalin 0.2 cc.....	130	84	1:00	Adrenalin.....	64	0	1:19
Adrenalin.....	132	84	1:03	Adrenalin.....	78	20	1:27
Histamine 0.04 mg.....	128	-50	1:05	Adrenalin.....	80	10	1:35
Histamine.....	124	-50	1:08	Adrenalin.....	78	12	1:44
Barium chloride 15 mg..	120	28	1:11	Adrenalin.....	80	18	1:49
BAL 0.5 cc. intravenous	132	-110	1:18	Histamine.....	72	-52	1:52
Adrenalin 0.4 cc.....	22	22	1:25	Benadryl 0.1 gm. intra-			
Adrenalin 0.2 cc.....	44	32	1:33	venous.....			
Adrenalin.....	34	24	1:45	Benadryl 0.1 gm.....			
Adrenalin.....	30	22	1:49	Adrenalin.....	74	12	2:18
Adrenalin.....	42	26	1:53	Histamine.....	80	0	2:20
Adrenalin.....	68	22	1:09	Barium chloride.....	80	10	2:22
Adrenalin.....	54	8	1:16	Adrenalin.....	106	14	2:30

strable on isolated blood vessels. To this end, the vessels of the isolated rabbit's ears were perfused with phosphate containing Ringer's solution and Benadryl along with the stimulating substance injected into the stream of perfusing fluid.

The results (table 3) show that the response to histamine, adrenalin, tyramine,

angiotonin and rabbit's serum are all significantly reduced or abolished. Serum vasoconstrictor (Serotonin) and adrenalin were found the more difficult to block. Comparable results were obtained when the ear was perfused with diluted normal dog plasma. That the blood vessels were not irreversibly poisoned by the Benadryl is shown by the fact that within a few minutes, responses equal to those of the control injections returned.

Fluid Exchange: Intact Animals. When the pressure in a reservoir containing 0.9 per cent saline connected with the femoral artery is equal to or slightly above arterial pressure, little fluid enters the circulation in normal dogs. In some normal appearing animals, however, the uptake from the reservoir may be as high as 500 cc. over a period of an hour or more, but this is exceptional.

Whether animals in which vascular refractoriness had been elicited by Benadryl and BAL would take up more than normal amounts of fluid was next determined.

TABLE 3. EXAMPLE OF EFFECT OF BENADRYL ON CONSTRICTION PRODUCED BY INJECTION OF VASOCONSTRICTORS INTO THE BLOOD VESSELS OF ISOLATED RABBIT'S EAR, PERFUSED WITH PHOSPHATE-RINGER'S SOLUTION

SUBSTANCE INJECTED	CONC.	VOL.	DROP RATE REDUCTION ¹					
			Before Benadryl		With Benadryl (0.2 cc.) ²		After Benadryl	
	mg./cc.	cc.	min.	%	min.	%	min.	%
Rabbit serum		.2	6	78	1	35	5½	74
			6½	82			7	88
Epinephrine	.005	.2	15	99	1¼	60	8½	86
Histamine	.02	.2	6½	83	0			
Tyramine	.02	.2	13	88	0			
Angiotonin		.4	4	29	0			

¹ Original drop rate 40/min.

² 0.1 mg/cc. Benadryl.

It was immediately apparent that such animals took up grossly abnormal amounts, and their time of survival was shortened. It was as though the least pressure on the vascular tree caused it to dilate and make room for large amounts of fluid, or that the permeability had so increased that the fluid immediately ran out into the tissues.

Since we had found that burn shock produced vascular refractoriness, some of these experiments were repeated with the additional measurement of the fluid exchange. Under deep anesthesia, the hind and fore quarters of the animal were dipped in boiling water for 2 minutes. At no time was the anesthesia lightened before death.

The response to adrenalin, histamine and barium chloride after scalding was roughly one fifth that before. One example will be given to illustrate the group of experiments. For 3 hours, even at a pressure within the saline-containing reservoir connected to the femoral artery of 160 mm. Hg, blood flowed slowly out of the animal into it. Then flow was reversed and ever greater amounts of saline mixed with blood flowed back into the animal until during the last 40 minutes of the animal's life, the

rate reached the high value of 1512 cc./hr. The total uptake was only 2722 cc. despite the high pressure initially maintained in the reservoir.

Another experiment which seemed comparable as to survival time was selected to illustrate some differences in fluid exchange when Benadryl was given before and

TABLE 4. EFFECT OF SCALDING ON THE PRESSOR-DEPRESSOR RESPONSES AND FLUID INTAKE (EXP. 29)

SUBSTANCE INJECTED	TIME, HOURS:MIN.	INITIAL BLOOD PRESSURE	RISE OR FALL	SALINE EXCHANGE, ¹ CC. PER HOUR
Adrenalin	:00	162	82	
Histamine	:00	168	-48	
Barium chloride	:00	160	22	
Scalded				
Adrenalin	:15	154	32	
Reservoir opened 160 mm. Hg				
Adrenalin	1:15	172	34	-110
Adrenalin	2:15	132	12	-20
Adrenalin	3:18	116	16	-140
Adrenalin	4:18	100	14	30
Adrenalin	5:20	100	18	150
Histamine	6:22	112	-28	
Barium chloride	6:33	114	16	516
Adrenalin	7:40	112	18	784
Adrenalin	8:30	110	24	1512

¹ Fluid uptake by the dog.

TABLE 5. EFFECT OF SCALDING ON THE PRESSOR-DEPRESSOR RESPONSES AND FLUID EXCHANGE OF A BENADRYL TREATED DOG (EXP. 32)

SUBSTANCE INJECTED	TIME, HOURS: MIN.	INITIAL BLOOD PRESSURE	RISE OR FALL	SALINE EXCHANGE, ¹ CC. PER HOUR	SUBSTANCE INJECTED	TIME, HOURS: MIN.	INITIAL BLOOD PRESSURE	RISE OR FALL	SALINE EXCHANGE, ¹ CC. PER HOUR
Reservoir opened at 110 mm. Hg									
Adrenalin	:00	142	70		Histamine	:52	112	-20	-60
Histamine	:00	136	-58		Adrenalin	1:54	110	40	308
Benadryl					Adrenalin	2:54	108	48	284
Choline	:00	140	-42		Benadryl				
Histamine	:00	138	-16		Choline	3:54	112	4	579
Choline	:00	134	-12		Adrenalin	4:59	112	48	500
Scalded					Adrenalin	7:10	108	34	720
Adrenalin	:18	112	72		Adrenalin	8:17	106	26	1133
Histamine	:20	106	0						

¹ Fluid uptake by the dog.

two hours after the scald. The response to adrenalin was somewhat better maintained but the histamine and choline responses were greatly damped. The total fluid uptake, despite the lower pressure in the reservoir (110 mm. Hg) was 3464 cc., which is 742 cc. more than in the example of the dog untreated with Benadryl. Eight experiments showed the same trend. When both Benadryl and BAL were administered, fluid uptake was further enhanced.

DISCUSSION

The problem of refractoriness of blood vessels to stimuli has received little attention. We have been at some pains to point out that tachyphylaxis and refractoriness are different phenomena, though easily confused (8). Repeated administration of renin quickly leads to tachyphylaxis and its appearance is dependent on the repetition of the dose of renin. Angiotonin, the product of the action of renin on renin-substrate, on the other hand, may be given many times without reduction of response. But, if the central nervous system of the test animal is injured or the animal is thrown into shock by burn or tourniquet, then the response diminishes or is lost. The reduced response in this case is not dependent on the repetitive administration of the stimulant.

Vascular refractoriness, not tachyphylaxis, is the concern of this communication. Doubtless the former can be produced by many substances, but we have chosen Benadryl because of its known antihistaminic action and BAL because of its ability to block the action of a variety of enzymes. As it soon became evident, both substances affect many vasoactive substances and their administration, together in large doses, produces refractoriness, in some cases complete, to such agents as adrenalin, angiotonin, tyramine, choline, histamine and barium chloride. Despite the loss of responsiveness, the arterial pressure may be maintained at normal or even elevated pressures for half an hour or more to be followed without warning by vascular collapse.

The action of Benadryl and BAL in producing refractoriness seems to be due chiefly to their direct action on blood vessels because the same type of blocking occurs at least with Benadryl in the vessels of the isolated rabbit's ear.

The capacity of the vascular tree appears to be increased when vascular refractoriness is elicited. This is deduced from the observation that the uptake of saline from a reservoir maintained at a constant pressure, but initially at or above arterial pressure, is considerable an hour or more after severe scalding. An alternative or correlative explanation is that vascular permeability increased and saline flowed much more rapidly out of the blood vessels. Our experiments do not indicate which explanation is the more valid.

When Benadryl was administered to add to the vascular refractoriness elicited by scalding, the uptake of fluid appeared to be further increased. So far as we could tell in experiments with so many variables, survival time of the dogs was not reduced. But if both Benadryl and BAL were given before the scald and refractoriness was all but complete, the uptake of fluid was greatly enhanced and survival time greatly reduced. The severe vascular refractoriness so elicited must have significantly increased vascular capacity by dilatation, increased vascular permeability or both.

SUMMARY

Refractoriness to chemical stimuli has been produced in dogs by administration of large doses of Benadryl and BAL. Arterial pressure may be well maintained for 30 minutes or more, but circulatory collapse follows. This phenomenon may be an artificial prototype of the refractoriness occurring in shocked animals. Refrac-

toriness is probably in part due to direct action of Benadryl on the blood vessels because when perfused along with chemical stimulants through the vessels of isolated rabbits' ears, vasoconstriction is significantly reduced or abolished. Refractoriness reduces the ability of the vascular tree to withstand increased pressure transmitted to it from a pressure reservoir containing normal salt solution as demonstrated by rapid uptake of fluid. Similarity is found in the response after severe scalding, for here too, as refractoriness increases, uptake of fluid accelerates. Benadryl and BAL given in sufficient doses to produce refractoriness further augments the effect of scalding in that fluid uptake increases and survival time decreases.

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VARIATIONS OF VASCULAR REACTIVITY IN NORMAL AND HYPERTENSIVE DOGS

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RESPONSES of blood vessels to stimuli—the vascular reactivity—may be measured in a variety of ways; in this investigation the pressor-depressor response to intravenously injected drugs has been chosen. This is convenient. Also, the suggestion has been made recently (1) that experimental hypertension might be initiated by a renal pressor substance, but the hypertension being maintained in a matter of weeks by the nervous system, so relegating the humoral mechanism to insignificance.

The evidence for this view is of three sorts. The first is that increased pressor reactivity occurs directly after induction of hypertension to a variety of vasotropic agents, such as adrenalin and pituitrin, the increased sensitivity preceding actual elevation of arterial pressure. After several weeks, reactivity decreases. Pentobarbital, which presumably acts chiefly on the nervous system, exhibits little tendency to lower blood pressure initially, but as the adrenalin response decreases, that to pentobarbital increases. The second is that examination of peripheral blood seems to show renin to be present in measurable amounts during the first week of experimental hypertension, only to disappear later. The third is that the early removal of the presumed hypertension-producing kidney cures the hypertension but late removal has little or no effect.

If substantiated, the concept would have an important bearing on further studies of vascular disease. But so far support for the view is conflicting. Several years ago, Page (2) found no consistent difference in the pressor response to angiotonin before and after induction of hypertension by means of silk perinephritis or clamping the renal artery. It seemed desirable to extend this investigation to include study of vascular reactivity to substances other than angiotonin. Further, it has become abundantly evident that the response to pressor and depressor drugs often varies widely in an entirely unpredictable fashion, not only from day to day, but from hour to hour. We have therefore gone to some pains to make the conditions under which the measurement of vascular reactivity as uniform as we could. The purpose of this paper is then to examine, under carefully controlled conditions, the response to vasoactive agents, some acting more centrally, others more peripherally, before and after the development of experimental renal hypertension.

METHODS

Arterial pressures were recorded by means of a mercury manometer on a smoked drum. Heparin was used in the tubing and the femoral artery was cannulated. All injections were given into a

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femoral vein. The concentrations and doses of the various stimulating agents were: 1) 0.2 cc. adrenalin 1:10,000 dilution; 2) 0.15 cc. nicotine 1:1000 dilution; 3) 0.04 mg. histamine phosphate; 4) 0.5 cc. barium chloride 18 mg/cc.; 5) 0.5 cc. angiotonin 10 U/cc.; 6) 0.2 cc. renin; 7) 10 mg/kg. body weight of tetraethylammonium chloride, TEA (Etamon; Parke, Davis and Co., 100 mg/cc.).

Some of the dogs were tested with and without anesthesia, but most received the usual dose of 35 mg/kg. of pentobarbital sodium intraperitoneally and the test conducted from one to one and a half hours after the injection. Hypertension was elicited by the silk perinephritis method of Page (3) in some cases both kidneys being wrapped and in others one kidney wrapped and the other removed.

RESULTS

Change in Vascular Reactivity During Single Experiments and Different Days in the Same Animal

It was first of importance to know the degree of spontaneous change of vascular reactivity during the course of a single experiment. The results in table 1 show the rather wide changes occurring with nicotine and the less variant ones of adrenalin, histamine and barium chloride. Some of the differences for any of these drugs may be wide and demonstrate, we believe, the unreliability of accepting single tests as representative of the average vascular response. For example, in tests on 267 normal dogs, the initial response to adrenalin averages 10 mm. Hg less than succeeding doses.

Taken in normal dogs on different days over periods of months, much wider changes in vascular responsiveness occur (table 2). Thus in experiment 414, table 2, the adrenalin response varied from the extremes of 20 to 58 mm. Hg; nicotine from -84 to +42; angiotonin from 16 to 28; and barium chloride from 10 to 22 mm. Hg. No relationship between response and the initial height of the arterial pressure was observed. Thus, unless an average response is obtained, confirmed over a reasonable period of time, individual determinations of vascular reactivity may be grossly misleading.

It is of especial interest that tetraethyl ammonium chloride (TEA) which blocks autonomic ganglionic transmission (4) and is thought to measure autonomic participation in maintenance of the level of arterial pressure, produces an even wider variety of response on different days, ranging from definite lowering to actual elevation of blood pressure. This seems strong evidence that factors controlling blood pressure must vary significantly not only from minute to minute but from week to week, as suggested by Levinson, Reiser and Ferris (5).

Average Vascular Responsiveness in Anesthetized Dogs

In spite of the wide variability in response by repeated tests on a large number of dogs, it is possible to obtain an average value which is demonstrably useful in comparison with values obtained after experimental treatment of the same animals. The results of the examination of 65 dogs one to two and a half hours after intraperitoneal administration of pentobarbital (35 mg/kg. body weight) with an average weight of 9.8 kg. show:

AVERAGE RESPONSE OF 65 ANESTHETIZED (PENTOBARBITAL) DOGS TO VASOACTIVE AGENTS

	ADRENALIN	NICOTINE	HISTAMINE	BAR- IUM CHLORIDE	RENIN	ANGIOTONIN	TEA
Change in arte- rial pressure.	+45	+42	-42	+22	+38	+17	+10-38

TABLE 1. VASCULAR REACTIVITY THROUGHOUT SINGLE EXPERIMENTS

EXP.	ADRENALIN	NICOTINE	HISTAMINE	BaCl ₂	B.P.	TIME AFTER ANES- THESIA GIVEN	
						<i>min.</i>	
408	42	22	-36	10	180	42	anesthesia light
	32				170	20	
	26	28	-34	20	164	15	
	28	62	-22	16	136	160	
	28	10	-32		140	88	very light
	28	12		14	138	20	
		10	-20	16	146	56	very light
418	16	36			178	60	
	12	28		24	180	30	
		46			176	20	
416	88	44			160	30	
		58			186	20	
		62		8	182	30	
342	32		-28	24	132	90	
	50				120	18	
	26		-20	18	114	59	
353	28	-10	-44	8	152	65	
	36	-10+6	-40	4	140	66	
	36	-36+28	-52		120	70	
	30	-22+22	-54		116	48	
	30	-40+18	-60		128	61	Pentobarbital
	36	-38	-48		130	57	
	32	-18+12	-30		108	11	Pentobarbital
	16	-40		16	112	56	
414	26	42	-28	22	136	135	8/ 9/46
	20	18	-30		140	60	
	36	30	-30	30	168	120	
	46	26	-30		168	21	8/20/46
	28	34	-42	16	142	10	
	42	-56+18		10	128	100	9/30/46
	48	-62+12			124	30	
	38	-84	-36		126	60	
	44		-30		132	20	
	58				126	20	
	38				120	10	12/ 2/46

Vascular Reactivity Before and After Induction of Hypertension

It was of especial importance in these experiments to have sufficient numbers of control responses before the hypertension was elicited. At least some, although by no means all, of the variable factors which confront such inquiries are eliminated by the

method of using the same animal both as normotensive control and hypertensive subject. In table 3, the average values are given before, during the development and after the hypertension had been well established. In some animals, the tests were repeated within a few days or weeks of applying the wrappings to the kidneys; in others only when hypertension was present for several months.

If allowance is made for the variability in vascular responsiveness which may occur spontaneously, it will be apparent that no trend is established in response to adrenalin, nicotine, histamine, barium chloride, renin, or TEA. Nicotine, as usual, showed the greatest variability, yet no relationship of response to the maintained average level of arterial pressure was evident. For example, in *experiment 418*, the response to nicotine on August 20, with blood pressure at 186 mm. Hg was 8 mm. Hg,

TABLE 2. VASCULAR REACTIVITY IN SAME DOGS OVER LONG PERIODS OF TIME

EXP.	ADRENALIN	NICOTINE	HISTA-MINE	BaCl ₂	ANGIO-TONIN	TEA	B.P.	WT.	DATE	
423	30	24	-46				140	12.3	2/ 2/47	
	66	30	-54				134	11.9	2/ 5/47	
	58	92	-42			-66	130	11.2	2/18/47	
511	+10-20	-14+18	-46				160	10.1	1/24/47	no anesthesia
	12	18	-62		16	-48	140	10.3	2/10/47	
	28	86	-52		26	+28	122	10.6	2/20/47	
	34	40	-42	72	22	-42	102	11.9	3/ 5/47	
	48	40	-50	44		+38	160	14.5	9/ 9/47	
414	26	58	-28	22			138	13.6	8/ 9/46	
	36	34	-30	30			168	13.6	8/20/46	
	42	-56+18		10			128	13.8	10/30/46	
	38	-84	-36				126	13.9	12/ 2/46	
536	26	8	-50	14	22	+10-18	136	11.9	3/ 7/47	
	56	10	-36	14	28	+32	118	13.9	6/27/47	

while on November 11, with almost the same pressure, it was 102 mm. Hg. This might suggest that the nervous system had become persistently highly responsive during the period, were it not for experiments such as 98 and 186. Here the nicotine response changed only from 20 to 26 mm. Hg despite the fact that blood pressure rose from mean of 140 to 196 mm. Hg. There seems to be no discernible relationship between either the height of the blood pressure or the time after hypertension has been elicited and the response to the pressor-depressor agents employed.

Critique of the Method. Factors Possibly Influencing Vascular Responsiveness

Length of residence in kennel. The responsiveness of common street dogs usually kept in the kennel a week or more was compared with others which had lived there a good part of their lives. We were unable to detect any regular difference in the responses of these two groups of animal.

Temperament of the animal. When only procaine hydrochloride anesthesia was

employed during cannulation of the artery, the temperament of the animal would be expected to make a great difference in the drug response. Consistent differences were largely limited to the nicotine response, which usually was greater in excitable

TABLE 3. VASCULAR REACTIVITY BEFORE AND AFTER INDUCTION OF HYPERTENSION

EXP.	ADREN- ALIN	NICO- TINE	HISTA- MINE	BaCl ₂	ANGIO- TONIN	RENIN	TEA	B.P.	WT.	DATE	
104	86						+14-58	146	9.5	2/14/47	Control
113	28	6	-76	20			-50	140	9.3	2/24/47	Control; lt. kidney wrapped and rt. nephrectomy
127	38	32	-88	14			+22	180	6.1	3/12/47	Killed
106	26							140	13	2/17/47	Control
122	8	0	-44	16	12			140	12	3/6/47	Control; lt. kidney wrapped
129	28	4	-42	10	14	38	-38	120	11.8	3/17/47	Rt. kidney wrapped 4/9/47
196	+16-28	12	-36	14		24	+14-14	170	12.4	6/3/47	
398	54	16	-28	15				148	13.2	8/9/46	Control; both kidneys wrapped
	30	52	-56	10				200	12.3	11/13/46	
	60	48	-74	32				192	12.3	11/13/46	
	42	44	-44					150	11.5	1/25/47	
	66	7200	-22	28			-52	190	11.8	2/21/47	No anesthetic used
	62	38	-44	26	30	28	-12	204	14.2	4/9/47	
98	26	20	-38					140	14.7	1/28/47	Control; both kidneys wrapped.
101	36	22	-58				-22	116	13.2	2/4/47	
186	+18-22	26	-40	10	12	14	+14-52	196	14.0	5/17/47	
197	26	34	-30	20	4	14	-42	160	15.0	6/3/47	
418	26	28		24				124	9.2	8/8/46	Control; both kidneys wrapped
	20	8	-36	12				186	9.1	8/20/46	
	60	102	-76	24				184	9.0	11/4/46	
576	30	10	-18	8		42	+24-10	110	8.4	3/29/47	Control
	54			18		64		116	8.2	4/2/47	Both kidneys wrapped
	90	+30-28	-44	32		94	+70	174	8.6	6/27/47	
	70					46					
570	34	22	-30	20			-72	110	10	2/24/47	Both kidneys wrapped; no hypertension developed
	72	26	-42	22		24	+12-24	112		3/12/47	
181	40	-28+20	-18	20		34	+30-30	150	7.6	5/13/47	Control
	94			18							
198	80	34	-30	24		46	-88	190	7.7	Rt. kidney wrapped 5/13. Tested 6/4	
	132			60		44					
250	26	34	-36	36	16	20	+26-66	220	7.9	8/8/47	
	70		-50	50		42					

animals. The stimulation of respiration elicited probably participated in making the animals apprehensive. Often responses even greater than those without anesthesia were observed under pentobarbital anesthesia.

Weight of animal. A careful attempt was made to demonstrate a direct relationship between the weight of the animal and amount of drug used as stimulant but with little success. It was therefore abandoned in favor of standard doses. The excep-

tion was TEA with which the standard dose of 10 mg/kg. body weight was used. Even here it is not certain that the results were more uniform.

Depth of anesthesia and initial blood pressure. We were able to confirm our earlier observation (2) that within reasonable limits, the depth of anesthesia and the initial height of the blood pressure did not influence responses in a regular fashion. In a few dogs, the pressure was held at various levels by the method of controlled bleeding (6). The responses when the pressure ranged from 80 mm. Hg to 160 mm. Hg showed no consistent trend.

Character of the blood pressure response. The order in which these drugs were given, provided the blood pressure had returned to control levels before the next drug was given, did not affect the results. Nevertheless, the plan was adopted of giving them in the following order: adrenalin, nicotine, histamine, barium chloride, angiotonin, renin and TEA.

We have repeatedly noticed that a second dose of adrenalin at the beginning of the experiment usually produced a greater response than the initial one. At times, the latter was biphasic, so reducing the total rise in pressure. This could be overcome in most animals by repeated administration of the drug. This has been done before accepting the control reading. These deviations from the more usual pattern point to the need for recording the form of induced changes in arterial pressure. Methods in which the pressure change is read off a manometer from time to time, such as those commonly used in unanesthetized rats and rabbits, would almost surely miss such deviations, thus yielding false values. We believe, therefore, that correct evaluation of the induced changes in blood pressure is dependent on recording these changes to ascertain that the response is not a deviant one.

Tubocurarin. Since the test was a painless procedure, it was hoped that relaxation could be secured by intravenous tubocurarin, so avoiding general anesthesia. The amounts given and examples of this kind of experiment are shown in table 4. An attempt was made to produce either a gradually increasing paralysis or a very rapid one. Neither method aided in increasing the uniformity of response; rather the reverse was true.

Local anesthesia. Seven dogs with widely varying temperaments were studied with only local procaine-hydrochloride injection to prevent pain during cannulation of the artery. The differences in apparent emotional state are indicated in table 5. The adrenalin responses, although more variable, resembled in intensity those under general anesthesia. Nicotine responses were certainly greatly increased and unusually variable while renin, angiotonin and barium were only somewhat less variable. To our surprise, dog 111, which had always shown a docile and quiet temperament, exhibited a rise of more than 200 mm. Hg with the standard dose of nicotine, which is 150 mm. Hg more than in normal anesthetized dogs of the control group showed. The most widely fluctuant response, however, was that to TEA, which ranged from a fall of 66 mm. Hg to a rise of 30 mm. Hg and showed no apparent relationship to the outward appearance of calmness of the dog. In a few experiments, for example, dog 353 (table 1), after repeated testing without anesthesia, pentobarbital was given and the testing repeated. It is surprising how little, rather than how much, the responsiveness changes.

TABLE 4. EFFECT OF TUBOCURARIN PARALYSIS ON VASCULAR RESPONSIVENESS

EXP.	TUBOCURARIN	TIME AFTER	ADRENALIN	NICOTINE	HISTAMINE	ANGIO-TONIN	B.P.	
	cc.	min.					mm. Hg.	
93	1.0	15	0	42	0	18	182	Animal now pithed partially
		20	14	38		14	140	
		9	12	0			60	Arterial transfusion of saline Pressure at 64 mm. Hg
		10	2				44	
		5	18				64	
		3	24	32		4	64	
		15	36	30		3	64	
97	2.0	10	+12-18				146	
	0.2	10		160			164	
	0.2	10	22		-20	22	134	
106	0	—	26				140	
	0.4	2	44				130	
	0.3	6	10	38			62	
94	0	—	26	32			88	49 minutes after bilateral adrenalectomy under ether. No anesthetic required for exp. Artificial respir. started
	0.7	6	52	56			55	
		6	38			38	66	
		7	46	46			36	
		11	34	46			50	Both vagi cut Arterial transfusion of saline at 50 mm. Hg
		3	34	16		20	40	
								Both carotids tied

Example of the Effect of Temperature Change

EXP.	RECTAL TEMP.	TIME	ADRENALIN	NICOTINE	HISTAMINE	BARIUM CHLORIDE	B.P.	
		min.						
443	107.6	0	22	22	-46	6	124	Anesthesia light
	111	67	34	22	-44		122	
	110	48	28	46	-38		130	
	112	90	30	48	-44		134	
	101	56	28	56	-48		130	
	100	49	30	38	-46		130	
	99.6	27	18	18	-36	30	128	

TABLE 5. LOCAL ANESTHESIA (NOVOCAIN) AND VASCULAR REACTIVITY

EXP.	ADRENALIN	NICOTINE	HISTAMINE	BARIUM CHLORIDE	ANGIO-TONIN	RENIN ¹	TEA	B.P.	CONDITION
107	22					54	-24	172	Excited
108	58	92	-42			60	-66	132	Calm
109	16	88	0		10	20	+30	160	Excited
110	28	86	-52		26	24	+28	132	Very calm
111	66	>200	-22	28	42		-52	190	Calm
153	34		-48	16		14	+8-46	102	Moderately excited
172	18	-46+28			20	8		124	Calm

¹ 0.1 cc. of renin instead of the usual 0.2 cc.

Effect of temperature change. A copper-lined cradle with heating coils was built so as to contain the animal's body within it. A thermocouple connected with the temperature control of the cradle inserted into the rectum controlled the temperature of the cradle by the desired temperature in the rectum. By this means wide, controlled variations could be induced.

Examples of these experiments are given in table 4. Changes from 100 to 111°F. produced no consistent change in adrenalin, histamine or nicotine response. Temperature appeared to affect the response hardly at all. Clearly, the small changes which occur during the usual test is not a deciding factor in the changing response. Rodbard (7) recently found no difference in adrenalin or acetylcholine response either in hypothermic or hyperthermic chickens, rabbits or turtles.

Muscular relaxation with myanesin. Berger and Bradley (8) showed that α - β -dihydroxy- γ -(2-methyl-phenoxy)-propane (Myanesin)¹ produces muscular relaxation and paralysis after its intravenous injection into animals without causing respiratory arrest.

Myanesin (100 to 150 mg/kg.) produced moderate relaxation with a curare-like effect which lasted 7 to 10 minutes. The vascular responses determined before and after the drug showed them to be slightly augmented by it. The usual increase from TEA was observed after its administration.

Use of myanesin was not continued because of the difficulty of keeping the paralysis and relaxation uniform. Further administration of the drug often led to vomiting and the extreme salivation made it difficult to keep the airway free. Greater doses than 150 mg/kg. usually caused the animal to become moribund.

Fluid administration. A femoral artery of a normal, anesthetized dog was connected to a reservoir containing normal salt solution or blood kept under a fixed air pressure as used by Kohlstaedt and Page for intra-arterial transfusions. The pressure in the reservoir was then raised almost 20 mm. above the systemic arterial pressure. Salt solution flowed into one animal, for example, at the rate of 300 cc/hour until 482 cc. had entered the circulation. Vascular responses were unchanged from the control levels. Large volumes of fluid may be taken up by some animals under these circumstances, while in others there is little or none. Irrespective of this factor, the result on vascular responsiveness was insignificant in these otherwise normal animals.

DISCUSSION

The closer examination of the method of assaying vascular reactivity has been rewarding since it shows how intricate the method is. Not only do changes in reactivity occur over periods of days and weeks but within hours and minutes. From the conceptual point of view, the constancy of the responses might be stressed since the wide variety of changes experimentally induced must have subjected the homeostatic mechanism of the body to great strain. But it is this as yet uncontrollable variability which makes difficult the interpretation of results obtained by use of such methods.

We have studied a variety of factors which might be concerned in the stability of vascular responsiveness. Among them are the length of residence of the street

¹ We thank Dr. F. M. Berger for a supply of myanesin.

dogs in the kennel, the temperament of the animal, the weight, the depth of anesthesia, the initial blood pressure, lack of anesthesia, internal temperature changes, artificial alteration of the level of arterial pressure, and the effect of relaxing agents such as tubocurarin and myanesin and finally, artificial changes in blood volume. None of these seems to be of decisive importance. Possibly the most significant observation is that pentobarbital anesthesia of moderate depth does not conspicuously alter the responsiveness to the test drugs with the exception of nicotine. Relaxing drugs such as tubocurarin and myanesin seemed to increase the variability of response rather than stabilize it. Of practical importance is the rather obvious but frequently neglected observation that unless the sweep of the blood pressure change is recorded, many important abnormalities such as diphasic responses are easily missed. In most studies on reactivity in rats and rabbits, serial unrecorded blood pressure measurements have been customary hence are subject to this error, an error which may be great.

Unless the normally wide variability in vascular responsiveness is weighed against experimentally-produced changes, wholly misleading conclusions may be drawn. When such a comparison is made in the same dogs before and after being made hypertensive by wrapping the kidneys in silk, it appears that neither early or late in the course of the hypertension is there any significant change in reactivity. As stimulants to measure reactivity, adrenalin, nicotine, histamine, barium chloride, angiotonin and renin run the gamut from largely centrally-acting agents to those acting chiefly on the vascular musculature. In 1941, Page (2) studied the same problem using angiotonin as the stimulating substance and similarly found no difference between normotensive and hypertensive dogs. During the next few years the stimulating hypothesis of Ogden (1) was presented. In essence, he suggested that the initiating mechanism of experimental renal hypertension was humoral, but after a period of weeks, the nervous system usurped the power of maintenance of the hypertension and presumably the humoral stimulators disappeared. The evidence is obtained chiefly from vascular sensitivity tests with pentobarbital, yohimbine, adrenalin and pitresin studied in rats and rabbits and using, for the most part, unrecorded discontinuous blood pressure measurement. Our earlier and present results together with recent ones of Moss and Wakerlin (9) do not support this hypothesis.

As we have pointed out before (10), testing of vascular reactivity in the manner all investigators have used does not rigidly exclude sensitivity changes. It is certain that the degree of response to one substance does not necessarily parallel that to another. Therefore, without employing the stimulating substance to which it is believed a change in sensitivity has occurred, generalization is unsound. Further, it is conceivable that augmentation of responsiveness is just sufficient to keep pace with increased humoral stimulation. The blood pressure rises but equilibrium is maintained between the stimulant, an augmentor substance, and the muscle of the blood vessel. Further stimulation by the injection of a drug would not show the animal's vessels more sensitive than normal.

The finding of Page and Taylor (11) that TEA greatly augments the responsiveness to angiotonin suggests this concept. If analogous augmentor substances occur naturally, and we assume this to be the case in the sensitization phenomenon follow-

ing bilateral nephrectomy, only enough might be produced to maintain equilibrium between humoral stimulant and receptive vascular muscle.

Cold pressor tests in animals with experimental renal hypertension have not shown any increased responsiveness according to Thomas and Warthin (12). This is probably another form of chemical stimulation mediated by the nervous system and so may properly be added to the evidence cited to support the view that significant changes in vascular reactivity are not demonstrable by the method employed. That the method is capable of demonstrating change in vascular reactivity under certain conditions is shown by observations (13, 14) in shocked animals. As shock deepens, the response to angiotonin and adrenalin progressively decrease, independently of blood volume and arterial pressure change. This can hardly be interpreted as anything but a loss of vascular reactivity.

SUMMARY

The response of the vessels to chemical stimuli as measured by arterial blood pressure change has been studied in 722 dogs. It was found to change during the same experiment and on different days. There was no direct relationship between the response to one drug compared with another, though often they paralleled one another. Nor was it a function of the height of the blood pressure, weight, temperament, length of kennel residence, depth of anesthesia, temperature, voluntary muscle relaxation, or intra-arterially administered salt solution.

Neither early nor late after eliciting renal hypertension does the vascular reactivity to adrenalin, nicotine, histamine, barium chloride, angiotonin, renin or tetraethyl ammonium chloride change in a characteristic fashion as to suggest the dominance of either the nervous system or a humoral mechanism in the production or maintenance of hypertension.

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INFLUENCE OF PROTEIN, CARBOHYDRATE AND SALT ON ARTERIAL PRESSURE OF DOGS WITH EXPERIMENTAL RENAL HYPERTENSION

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THE use of diets with widely varied protein, carbohydrate and salt contents for the treatment of patients with essential, malignant and nephritic hypertension came long before their validation by studies on experimentally hypertensive animals. But now that these are appearing, it is apparent that neither the results nor their interpretation present any uniformity.

Although observations made on dogs with experimental renal hypertension are not directly applicable to man, if only for lack of proof of its identity with essential hypertension, still they offer suggestive information. The results obtained on dogs with renal hypertension must be considered separately from those on rats with hypertension produced by desoxycorticosterone and salt (1) because of the probability that the mechanism of the hypertension is different. This communication is concerned only with the effect of dietary alterations on the arterial pressure of dogs with experimental renal hypertension.

The published evidence in favor of the view that when renal excretory efficiency is good, high protein diets, with or without alteration in salt intake, elevate blood pressure and low ones reduce it, is as follows. Chanutin (2) noted that in normal rats high protein diets caused renal hypertrophy but no increase in arterial pressure. In rats subjected to subtotal nephrectomy, some of the animals developed hypertension when fed meat extract. Verney and Vogt (3) found a surprisingly large increase in arterial pressure when dogs with hypertension were fed meat plus salt for 1 to 7 days, or meat to which large amounts of urea had been added. Similar changes were not observed in animals with normal blood pressure. Confirmation of these results was furnished from the study of one dog by MacLachlan and Taylor (4). Rises of 30 to 60 mm. Hg occurred on a meat diet when substituted for one of biscuits. Feeding of 50 gm. of urea was followed by hypertension of the same magnitude as that caused by the high protein diet. The addition of large quantities of salt to the usual diet of hypertensive rats does not elevate arterial pressure according to Grollman, Harrison and Williams (5).

The evidence suggesting that high protein diets do not have an effect on arterial pressure is more convincing. Phillipsborn, Katz and Rodbard (6) found a reversible rise in blood pressure in only 2 of 14 hypertensive dogs fed high protein diets, and in those only when renal insufficiency was present. Alpert and Thomas (7) could not demonstrate any effect of high protein diets in two dogs with renal and two with neurogenic hypertension. Similarly, Guerrant, Scott and Wood (8) were unable to influence the arterial pressure of two hypertensive and two normal dogs with high protein diets or administered urea.

Some of the conflicting results may be explained by the observations of Wood and Cash (9) who found that in hypertensive animals gain and loss in weight, regardless of the nature of the diet, is associated respectively with a rise or fall in systolic pressure. Probably most of the results cannot be so explained because the experimental diets were administered for too short a time to allow signifi-

cant weight changes to occur. Careful study of the published data strongly suggests that the lack of agreement is due chiefly to disregard for the wide variations in normal blood pressure. Another possibility is one suggested by Guerrant, Scott and Wood (8) that some dogs with clamps on the renal artery develop a less adequate capsular collateral circulation than others under the stimulus of large protein feedings.

These factors have been given due consideration in our experiments. First, the diets were fed for a sufficiently long period of time; second, the kidneys were wrapped in silk, thus preventing the development of collateral circulation; third, the trend of the average blood pressure level rather than a few measurements determined the decision as to whether the diet had an effect; and fourth, low protein, high carbohydrate diets were used as controls. The pattern of the plasma proteins was determined by the Tiselius electrophoresis method.

METHODS

Ordinary street dogs which had lived in the kennels for several months were used for the experiments. The diet consisted of dog biscuits and horse meat (one pound three times a week). Blood pressure was measured three times a week in a sound-proof room by direct femoral puncture. Several months were allowed for the heightened arterial pressure to become stabilized. The diets were prepared in such a way as to be roughly isocaloric in order to prevent in so far as was possible significant weight gains or losses. The high protein diet consisted wholly of raw lean meat, except in one experiment in which the meat was cooked. Hypertension was produced by wrapping both kidneys in silk or by removing one and wrapping the other (10).

The low salt diet consisted of one half pound of an almost sodium-free desiccated whole milk. This milk ('Lonalac', Mead-Johnson) supplies 145 calories/ounce and is composed of lactose 38 per cent; milk fat, 28 per cent; casein, solid, 27 per cent; calcium phosphate monobasic, 2.3 per cent; potassium carbonate, 1.9 per cent; potassium chloride, 0.7 per cent; thiamin, 0.36; niacin, 0.54, and riboflavin 0.72 mg/100 gm. The sodium content is 10 mg/100 gm. One half pound of Lonalac was mixed with cool water into a thin paste. The animals usually ate all of this, receiving 1135 calories per day.

RESULTS

High Protein and High Carbohydrate Diets

The results of 4 experiments were so consistent that the detailed data on the blood pressure of only one are presented (figure 1). Hypertension was established in this animal for 5 months while a mixed kennel diet was given. When all animal protein was withdrawn and carbohydrate substituted for the next 3 months, significant changes in blood pressure did not occur. The diet was so adjusted that the weight did not change. During subsequent months, the animal received an all meat diet, again followed by withdrawal of meat, but this time allowing less carbohydrate and more fat. Arterial pressure remained unchanged as it did on resumption of the regular mixed diet.

The results of the study of a second dog are worth recording because hypophysectomy had been performed 3 years and ovariectomy two years before the feeding experiment. A high protein diet was given for 3 months without change in average blood pressure. This was followed by a low protein, high carbohydrate diet. Despite a gain of 8 kg. of weight the blood pressure during the course of the next 3 months fell to definitely lower levels. An exploratory laparotomy showed cirrhosis of the

liver. The pathological anatomical findings on this animal have been described in detail by Graef, Negrin and Page (11) as *dog 1*.

The liver showed focal atrophy, early periportal fibrosis and marked glycogen storage. Glycogen storage and hyperemia were the only changes seen in the kidneys. There were none of the evidences of ischemia. The adrenal glands were especially interesting in that the medulla was relatively atrophied with apparent increase in the amount of cortical tissue. In most sections, Dr. Graef noted an adenomatous appearance.

In another dog, hypertension was induced by silk 5 months before feeding for 6 weeks a high protein diet of cooked meat. The blood pressure averaged 15 mm. Hg higher while on the diet than the control level. The blood urea nitrogen 6 days after starting the diet rose to 48.3 mg. and fell 7 days later to 24.8 and one month later to 17.1 mg/100 cc.

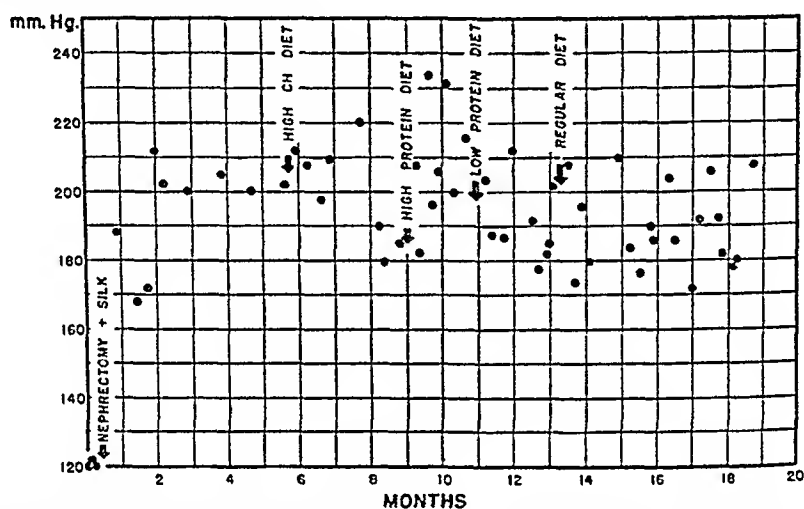


Fig. 1. EFFECT OF VARYING THE CARBOHYDRATE and protein content of the diet on a hypertensive dog's arterial pressure.

Low Salt Diets

Hypertension was well established in both dogs before the low salt diet was instituted. Then, over a period of 7 months, nothing but "Lonalac" was fed. Both animals lost almost half their weight. Nevertheless, arterial pressure was unaffected by the severe reduction in salt intake or weight. The fall in pressure of one dog (576), in the last month before death may have been due to rapidly declining health of the animal. The cornea became steamy, the hemoglobin fell to one third normal and blood-urea nitrogen rose to 149 mg/100 cc. on the last day of life. The results of the chemical examination of the urine testify to the fact that the diet was excessively low in salt. The urinary sodium ranged from 12 to 65 mg. in 24 hours, the variation being chiefly due to inability to collect exact 24-hour specimens. Repeated determinations of serum chloride and sodium showed no significant changes. In the other dog (550), the diet did not affect arterial pressure.

Electrophoretic Plasma Protein Patterns

The total plasma protein concentration of the hypertensive dogs while on the salt-free diet was higher than that observed in other hypertensive animals fed Purina

Dog Chow and horse meat. The average total protein of 20 hypertensive dogs on the regular diet was 6.66 ± 0.17 gm./100 ml. plasma, while the two hypertensive animals after 2 months on the salt-free ration were 10.22 and 8.31 gm/100 ml. An increase of 2.3 gm/100 ml. in the total plasma protein of one normal dog was observed 4 months after the diet had been changed from the regular to the salt-free ration.

The plasma gamma-globulin concentration of hypertensive dogs fed either diet was much higher than normal, 1.42 ± 0.08 gm/100 ml., i.e., 21.3 ± 1.0 per cent of the total protein in the hypertensive dogs on the regular diet, 3.27 gm. and 1.66 gm., i.e., 32 and 20 per cent in the hypertensive dogs on the salt-free diet. The average gamma-globulin of normal dogs (72 animals) fed the regular diet was $0.49 \pm$

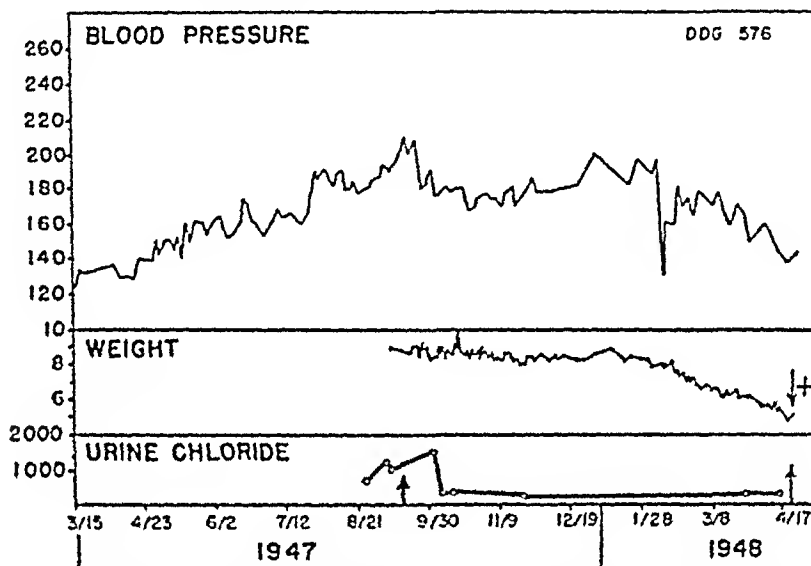


Fig. 2. EFFECT OF A DRASTIC LOW SODIUM DIET on a dog with renal hypertension. The animal died in uremia at the end of the experiment with great weight loss.

0.02 gm/100 ml., i.e., 9.1 ± 0.03 per cent of total protein. The gamma-globulin of the one normal dog on the salt-poor diet was 0.87 gm/100 ml., i.e., 11.4 per cent of the total protein.

DISCUSSION

The results of these experiments indicate clearly that even extreme changes in the protein and carbohydrate content of the diet fed for several months do not produce significant changes in blood pressure providing weight changes are not too great and, more importantly, that the dog maintains good health. What the effect would be if fed such ill-balanced diets over a lifetime remains unanswered.

Whether the occurrence of cirrhosis of the liver in the hypophysectomized dog was concerned with the fall in arterial pressure is not possible to determine from available evidence. In such animals, it would be of interest to measure the renin-substrate of the plasma.

The most startling observations made during the course of the investigation were that a large apical myocardial infarction had occurred in a young hypertensive dog. This probably accounted for a fall in blood pressure which occurred while the

animal was taking a low protein diet. The administration of large amounts of salt not only did not raise the blood pressure of this animal, but also did not increase the animal's weight presumably because loss of body tissue was occurring simultaneously. The dog died suddenly without premonitory signs. Besides the myocardial infarction, adenomatous hyperplasia was found in the adrenal glands.

The two dogs fed diets containing 25 mg. Na/day for a period of 8 to 9 months showed no significant change in arterial pressure, even though marked loss of weight occurred. It is reasonable to suppose that the reduction observed by other investi-

TABLE I. PLASMA PROTEIN PATTERN OF HYPERTENSIVE (NO. 576) AND NORMAL (NO. 1445) DOG WHEN FED A REGULAR OR SALT-FREE DIET

DATE	SPECIAL NOTES	TOTAL PRO- TEIN	ALBUMIN		GLOBULINS										Hb
			Gm/ 100 ml.	% of total pro- tein	α_1		α_2		β		γ		ϕ		
					Gm/ 100 ml.	% of total pro- tein	Gm/ 100 ml.	% of total pro- tein	Gm/ 100 ml.	% of total pro- tein	Gm/ 100 ml.	% of total pro- tein	Gm/ 100 ml.	% of total pro- tein	
<i>Hypertensive Dog 576</i>															
3-12-47	Normal—'regular diet'	5.40	2.38	44.2	0.55	10.1	0.29	5.3	0.94	17.4	0.43	8.0	0.81	15.0	14
4-2-47	Kidneys wrapped														
6-1-47	Hypertension es- tablished														
9-18-47	Salt-free diet started														
11-20-47	Salt-free diet	10.22	1.87	18.4	0.34	3.3	0.88	8.6	1.72	16.8	3.27	32.0	2.14	20.9	
2-6-48	Salt-free diet	9.08	1.73	19.1	0.55	6.1	0.69	7.6	2.22	24.4	2.23	24.5	1.66	18.3	7.5
4-20-48	Salt-free diet	8.00	1.19	14.9	0.68	8.5	1.11	13.9	0.62	7.7	2.10	26.2	2.30	28.8	<7.5
4-23-48	Died														
<i>Normal Dog 1445</i>															
6-19-48	Normal—'regular diet'	5.29	1.83	38.2	0.37	7.8	0.41	8.6	0.80	16.9	0.62	12.9	0.73	15.6	13.0
6-20-48	Salt-free diet started														
10-26-48	Salt-free diet	7.60	3.01	39.8	0.72	9.4	0.56	7.3	1.35	17.7	0.87	11.4	1.09	14.4	13.5

gators in a few animals was due to the normal variability of arterial pressure and to the brevity of the experiments. This however does not apply to the results of Dick and Schwartz (12) who found a fall to normal, or slightly above it, in 10 of 11 dogs with hypertension produced by intravenous administration of streptococci. The reduction occurred within 2 months after administration of the Kempner rice diet. An average weight loss of 20 per cent also was noted. The diet provided 13 gm. of protein, 212 gm. of carbohydrate and 900 calories if all the diet were consumed, which in many cases it was not. Although not measured, the sodium content of the diet must have been very low. This remarkable fall in arterial pressure has no counterpart in the dietary treatment either of experimental renal or essential hypertension.

The reason for the increase in total plasma protein concentration in the hypertensive and normal dogs on the salt-free diet is not clear. It occurred in the normal dog's plasma protein without significant shift in protein pattern and without evidence of hemoconcentration as judged by hemoglobin level. A relatively larger amount of 'complete' protein may have been available in the salt-free than in the chow-horse meat diet.

The relative concentrations of α_1 , α_2 and β globulins and albumin in the plasma of hypertensive dogs on the salt-free diet were similar to those observed in hypertensive animals on the regular ration. Later in the study, when the animals lost appetite, and relatively rapid weight loss occurred, the plasma albumin of one dog (576) decreased from 1.73 to 1.19 gm/100 ml., 19.1 to 14.9 per cent of the total protein.

The increase in γ globulin occurs during the development of hypertension. As previously noted (13) it is probably not due directly to reaction to the silk surrounding the kidney, for such increase is not observed when the spleen is so wrapped. The fall in albumin during the later part of the period that the dogs were on the salt-free ration was probably due to insufficient protein ingestion, and can be considered a direct effect of partial starvation.

SUMMARY

Four dogs with arterial hypertension of long standing induced by cellophane or silk perinephritis were given diets for several months either high in protein or low in protein and high in carbohydrate. Changes in arterial pressure which were considered significant were not observed in any of these animals. Fall in the blood pressure in one hypophysectomized hypertensive dog may have been associated with development of cirrhosis of the liver.

Fall of blood pressure in another hypertensive dog was associated with the occurrence of an extensive apical myocardial infarction which presumably appeared while the animal was receiving a low protein, high carbohydrate diet. Administration of 10 gm. of salt daily for 30 days did not elevate the blood pressure in this animal. Two dogs fed a diet of dried whole milk with the sodium largely removed ('Lonalac') for 7 months did not show significant changes in arterial pressure despite marked loss of body weight. Subsequent administration of 6 gm. of salt daily for 4 months did not appreciably affect arterial pressure.

Total plasma proteins on the salt-poor diet rose in hypertensive dogs due chiefly to increase in globulins. In the normal dog, all fractions increased.

We are most grateful to Mr. William West, Ralph Edmonds and Robert Parker for their skillful assistance, and to Mr. McGarrity and Dr. Bills of Mead-Johnson and Company for the 'Lonalac' used in these experiments.

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EFFECT OF HYALURONIDASE ON THE PASSAGE OF FLUID AND OF T-1824 THROUGH THE CAPILLARY WALL

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THE demonstration of the action of hyaluronidase as a spreading agent has been amply documented (1, 2). The effect of hyaluronidase on capillary permeability, however, has not been clearly defined. As a result of their microscopic observations on the omental capillaries of the frog, Chambers and Zweifach (3) regarded hyaluronidase "as a factor in accentuating capillary fragility rather than in inducing direct changes in capillary permeability." Several investigators (4, 5) have studied the effect of intradermally injected hyaluronidase on the diffusion into the skin of dyes administered intravenously. The effects of the hyaluronidase on the changes in the permeability of the capillaries depended on the action exerted on the outer surface of the vessel wall. Meyer and Ragan (6) noted that there was an increase in capillary permeability caused by hyaluronidase injected into the connective tissue, whereas intravenous injection had no such effect.

Duran-Reynals (4) studied the speed of passage of dyes from the blood into the tissues, when given with or without testicular extract. He noted that testicular extract accelerated the loss of dye, with the greatest accumulation of the dye in the foot pads and in the ears. Aylward (5) repeated these experiments in mice and rabbits, and observed that the experimental animals developed the skin color more quickly, and that the final color was more intense. An attempt was made to obtain a quantitative test by measuring the changes in the blood volume of the dog by use of the dye T-1824. However, no significant change was demonstrable with the amounts of testicular extract used.

The purpose of this investigation was to observe the physiological alterations produced by intravenously administered hyaluronidase, and to determine whether or not significant reduction occurred in the plasma concentrations of the dye T-1824, following the administration of hyaluronidase.

METHODS

One hundred male albino rats of the Sherman strain were used, varying in weight from 165 to 320 grams. They were fed the standard laboratory Purina chow and water ad lib.

Hyaluronidase Preparations. Fresh bull testes, stripped of fat and capsules, were ground up. An acetic acid extraction and several ammonium sulfate fractionations were carried out. The product was then dialyzed free of the sulphate and prepared in a saline-phosphate buffer at a pH of 5.3. It was stored at -20°C . It was assayed (7) and measured 2500 turbidimetric units/mg. of nitrogen.

A second testicular extract was prepared that was similar to the preceding, except that follow-

ing the ammonium sulphate fractionations it was further fractionated with acetone at -2°C ., dissolved in saline at a pH of 7 and stored at -20°C . It measured 3700 units/mg. of nitrogen.

The animals were anesthetized with sodium pentobarbital, 3 to 4 mg/kg., intraperitoneally. Two-tenths ml. of a 1 per cent aqueous solution of T-1824 was injected into the jugular vein. In 50 rats, 2500 units of hyaluronidase were injected through the same needle, and in 50 control animals an equal volume of 0.9 per cent saline was given.

At 5, 15, 30, 45 and 60 minutes, and at 2, 4, 8, and 24 hours, groups of experimental and control animals were bled and killed. Four to six ml. of blood were drawn from the abdominal aorta, using a heparinized syringe. Observations of the state of the organs were recorded. The blood was immediately centrifuged at 2500 r.p.m. for 30 minutes and the plasma separated from the cells. The concentration of the dye in the plasma was determined according to the method of Phillips (8).

RESULTS

Qualitative Observations

Diffusion of the dye into the tissues. Within 5 minutes following the injection of the hyaluronidase and dye in the experimental animals, the skin of the fore and hind legs, the nose, ears, mouth, tongue and wound over the jugular vein became discolored blue. This increased in intensity and reached a maximum in 30 to 45 minutes. The color began to fade within 4 to 8 hours and by 24 hours there was no discernible difference between the experimental and control groups. The control animals started to show very slight blue discoloration within 15 minutes, involving the foot pads of the extremities, the wound over the jugular vein and occasionally the nose.

Dye diffused into the abdominal wall, small and large intestine, walls of the renal pelvis, diaphragm, liver and, occasionally, the seminal vesicles, testes and retroperitoneal lymph nodes. The difference between the experimental and controls was not striking, although the former showed earlier and greater discoloration of these tissues. In none of the controls, was there extravasation of dye into the ears, tongue or periorbital regions. In neither group was dye found in the cerebrospinal fluid or in the urine.

Edema. The occurrence of edema approximated in extent, distribution and time of appearance that of the dye discoloration of the tissues. It was most marked in the extremities and the face, the former frequently being increased to twice their normal diameter. The skin of the dorsum of the paws was elevated several millimeters above the underlying tendons and bone; the foot pads bulged and the face had a puffy and coarse appearance. There was no demonstrable enlargement of the viscera, however. Within 24 hours the edema had disappeared in all of the experimental animals. In none of the control animals was any edema present.

Effect on blood vessels. Within 15 minutes following injection of the testicular extract, the arteries and veins had contracted to approximately one half of their normal diameter. The vessels involved were the aorta, iliac and renal arteries, and the veins of the abdominal cavity to a lesser degree. This persisted for several hours and disappeared in 4 to 8 hours. The abdominal viscera and extremities were cooler than normal, and the animals occasionally were shivering.

Quantitative Observations

The mean plasma dye concentrations in experimental and control animals are illustrated in figure 1. Within 15 minutes, the blood level of the dye was significantly

reduced in those animals receiving hyaluronidase, and this effect was most marked at 30 minutes. Thereafter, the difference between the two groups diminished. In 8 to 24 hours, there appeared to be no significant difference.

When these data were corrected for body weight of the rats, the same differences between the two groups were obtained. Although with statistical methods the validity of these results could be demonstrated, it was felt that insufficient numbers of animals were used to justify this type of analysis.

Several control experiments were performed in which the testicular extract was heated at 70°C for 30 minutes in order to inactivate the hyaluronidase. An *in vitro*

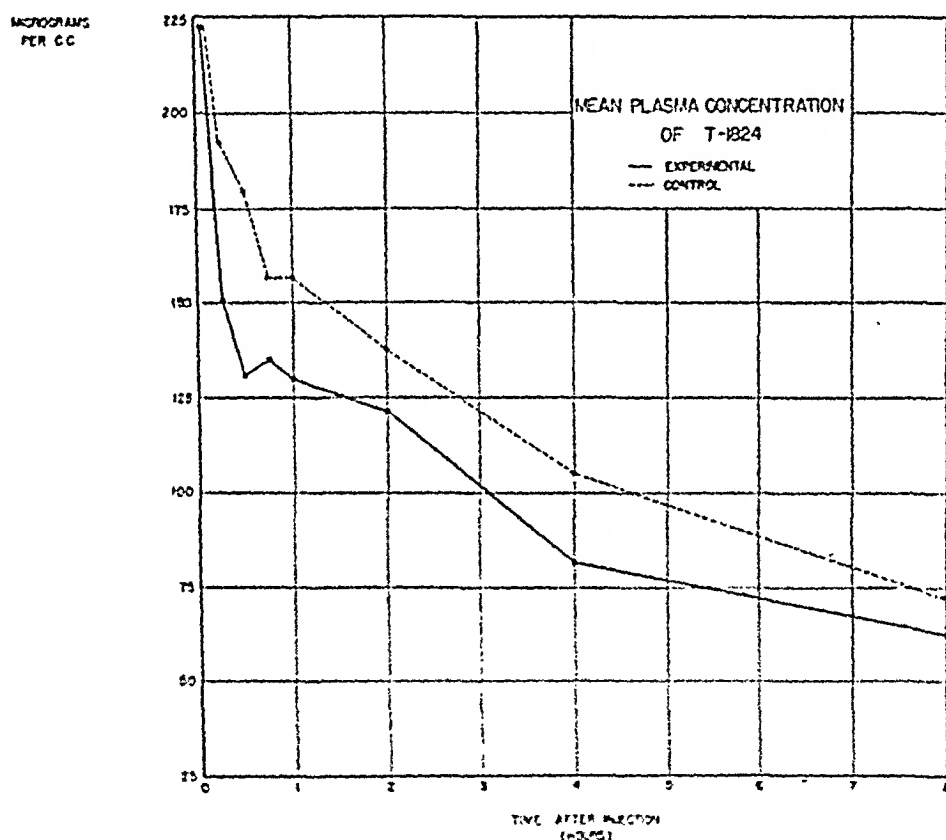


Fig. 1

assay confirmed the inactivation. When this preparation was given with the T-1824, the animals failed to become edematous and blue. To several animals, 6 mg. of crystalline bovine plasma albumin was given with the dye, and these animals also failed to demonstrate the aforementioned changes. One mg. of histamine was given to another group. Although these rats exhibited some tissue discoloration and a small amount of edema, it was small in comparison with that in the experimental group. In those instances that the plasma dye concentrations were determined, the results were similar to those found when saline and T-1824 were given.

DISCUSSION

These results confirmed those of Duran-Reynals and Aylward. The distribution of the dye in the tissues of the rat following the administration of testicular extract was similar to that which they described in the guinea pig, mouse and rabbit. Dye

diffused into the foot pads and face and particularly the ears, characteristic sites that were not involved in control animals. However, there was not any significant increase in dye concentration in the liver and lungs, as described by those two investigators. Experimental and control animals alike showed discoloration of these organs after 2 hours. The dye did not pass the cerebrospinal fluid barrier and did not appear in the urine.

The occurrence of edema has been very striking. Previous investigators have made no reference to this condition. The presence of edema, plus the diffusion of dye and the decreased plasma concentration of dye were evidence of an increased permeability of the capillary tree. The mechanism of action of the hyaluronidase may be by alteration or dissolution of the ground substance of the capillary wall. However, regeneration must occur rapidly, for the increased permeability was of short duration and appeared to be completely reversible.

The effects produced by the testicular extract appeared to be specific. The materials used in these studies were more purified than those used previously by other investigators. A few experiments carried out with hyaluronidase that contained 20,000 turbidimetric units/mg. of nitrogen, confirmed the results found with the cruder extract. Heat inactivated hyaluronidase failed to produce an increase in capillary permeability. That this was not just a foreign protein effect was demonstrated by the inactivity of bovine plasma albumin. Histamine did not produce an equivalent increase in the capillary permeability as measured by our techniques. Swyer (9) suggested that histamine was the basis of the skin-diffusing activity of hyaluronidase, but this appeared to be of little importance in these experiments.

SUMMARY

Following the intravenous injection of 2500 units of hyaluronidase and T-1824 into 50 male albino rats, edema and rapid diffusion of the dye into the extremities, the nose, ears, mouth and tongue occurred. There was contraction of the blood vessels and, at times, a shock-like syndrome. These changes were demonstrable within 5 minutes, were maximum in 30 to 45 minutes, and were completely gone within 24 hours. The plasma concentration of T-1824 was significantly reduced by the administration of the hyaluronidase. Hyaluronidase, when purified to 20,000 turbidimetric units/mg. of nitrogen, had the same effect of increasing capillary permeability. Hyaluronidase, when inactivated at 70°C. for 30 minutes, no longer was active. Bovine plasma albumin and histamine failed to produce an equivalent increase in the capillary permeability.

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URINARY FLOW AND EXCRETION OF SOLUTES DURING OSMOTIC DIURESIS IN HYDROPEMIC MAN

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THE osmotic limitations of renal functions in dehydration have been the subject of studies by various authors from different points of view. While Koranyi and other early workers studied extensively the maximum osmolality of urine in health and different pathological conditions (1), French authors (2) were interested in the urine flow in dehydration. They coined the term 'urine obligatoire,' a urine of high specific gravity and osmolality, which is excreted even in severe dehydration.

The factors controlling the concentration and volume of urine in hydropenia were not clarified in these or later studies. While some authors assumed the independence of excretion of electrolytes and urea (2, 3), with possible maximum concentrations of each co-existent, others found an inverse relation between their maximum concentrations (4). They suggested, therefore, that the total osmolality rather than that of any individual solute determined the maximum concentration and volume of urine in dehydration. Gamble (5), in studies on man during water deprivation, came to the conclusion that 1400 milliosmols per liter represented the maximum urinary concentration attainable. Given this fixed maximum, a minimal urinary volume, i.e. that volume of the 'urine obligatoire' could be calculated from the solute load. McCance and co-workers (6) adduced additional evidence that total osmolality rather than that of any component determined the maximum urinary concentration, and added information that with increasing flow during osmotic diuresis in hydropenia produced by urea, sodium chloride or bicarbonate, and potassium chloride, the osmolality of urine decreased.

The studies reported were undertaken with a goal of defining in a broad manner, by the use of loading solutes of various kinds, the limitations of renal function during conditions of water restriction. The present paper deals with the patterns of urine flow and concentration during osmotic diuresis.

MATERIALS AND METHODS

The subjects were male patients 8 to 15 years of age with normal renal function and without major disease. One group of diabetic boys was included for the study of forced diuresis produced by glucose. The loading substances were administered in amounts of 500 to 2000 milliosmols/1.75 m² body surface, by the oral or more often the intravenous route, in concentrated solution. The subjects were fasting and had received no water for 16 hours previous to the experiment and were excreting

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urine at rates of 0.5 ml/min. or less before loading (except the diabetic subjects). Eleven loading substances were studied. They were: glucose, sucrose, mannitol, sorbitol, sorbose, xylose, urea, creatinine, sodium para-aminohippurate, sodium sulfate and sodium chloride. In some experiments the entire course of diuresis, until the urine flow returned to preloading levels, was followed, in others chief emphasis was laid on observing the periods of maximum flow. Blood and urine were collected at appropriate intervals and were analyzed for the loading solute, as well as sodium, potassium, phosphorus, chloride, urea and, in some instances, sulfate. On urine the freezing point was also determined, and the 'true' total osmolarity calculated.²

Sodium and potassium were determined by flame photometry; phosphorus, according to Fiske and Subbarow (7); chloride, by the method of Van Slyke (8); urea, according to Van Slyke and Kugel (9), except in the creatinine loading experiments when the urease method of Van Slyke (10) was used; sulfate, according to Power and Wakefield (11), except for a final colorimetric measurement according to Klein (12), but under the conditions as described by Smith *et al.* (13); glucose, by the method of Nelson (14); sucrose and sorbose, by a modified method of Roe (15); mannitol and sorbitol, according to a modification of the procedure of Corcoran and Page (16); xylose, by a modification (17) of the method of Mejbaum (18); creatinine, by the Jaffé reaction, using a cadmium sulfate-sodium hydroxide precipitation (19); and para-aminohippurate, according to Smith *et al.* (13).

The administration of the loading substances was attended most commonly by complaints of thirst and headache of varying degree, which subsided promptly following the drinking of small amounts of water. Urea seemed to have a sedative effect, and sodium sulfate produced nausea, vomiting and urge to defecate.

² In calculating the osmolarity of urine the different freezing point behavior of electrolytes and non-electrolytes has to be considered. Whereas the molal freezing point depression of solutions of non-electrolytes remains constant at a value of 1.86° over a wide range of concentrations, the freezing point depression of electrolytes, identical with that of non-electrolytes at infinite dilution, falls progressively with increasing concentration. The observed deviation from theory increases with increasing valency of the ions concerned. Neglect of these considerations and use of a constant factor of 1.86° leads to an under-estimation of the osmolarity of the urine which is particularly great in sulfate loading experiments. In this report the true total osmolarity of urine was calculated from the freezing point depression and the concentrations of sulfate, urea, and the non-electrolyte loading solute, if present. The basic assumption was made that the divalent ions, the non-electrolytes and the univalent ions accounted for the total osmolarity of urine. It was further assumed that all solutes were univalent ions except for those determined as either divalent ions or non-electrolytes. Only the sulfate was considered among the divalent ions and only the urea and the loading solute, if present, among the non-electrolytes. In principle then the concentration of the univalent ions could be determined from the freezing point depression after first subtracting the contributions of sulfate and the non-electrolytes. For specimens on which sulfate was not determined, a sulfate value was assumed according to the minute volume as follows: min. vol/1.73 m² 0-1 cc., sulfate 25 m.Osm/l.; 1-2 cc., 10 m.Osm/l.; 2-3 cc., 6 m.Osm/l.; 3 cc., 3 m.Osm/l. The determination of the freezing point contributions was made possible by the close similarity of the molal freezing point depressions of the non-electrolytes, the various salts of univalent ions, and of the various sulfates with univalent cations, among themselves.

Therefore, one could use the value of 1.86 for all non-electrolytes and the appropriate molal freezing point depressions of NaCl and NaSO₄ (21) for the electrolytes. It was important of course to use in all calculations the concentrations of the solutes as extant in the aliquot of urine on which the freezing point was determined.

RESULTS

Thirteen subjects were studied in 15 experiments over 127 collection periods. Three illustrative experiments are presented in table 1. It includes data on the corrected minute volume of urine, the 'true' total osmolarity calculated as described in the experimental part, the sum of the solutes determined by analysis, the contri-

TABLE 1. URINE SOLUTES AND FLOW DURING OSMOTIC DIURESIS

PERIOD	CONCURRENT TIME	MIN. VOL./1.73 M ²	LOAD	TRUE TO-TAL OSMOLARITY	SOLUTES DETERM. BY ANAL.	SOLUTES DETERM.	LOADING SOLUTE I	LOADING SOLUTE II	LOADING SOLUTE
	min	cc.	m.Osm/min.	m.Osm/l.	m.Osm/l.	% of total	m.Osm/l.		% of total
<i>L.D., Diab.; 13 yr.; 38.9 kg.; S.A. 1.31 m²; Loading Substance—Glucose (oral and I.V.)</i>									
P-1	-91 to -61	1.02	1.190	1167	1111	95			
P-2	-61 to -38	0.86	0.988	1149	1129	98			
<i>-24 to 4 Glucose, oral, 366 m.Osm/1.73 m², I.V., 256 m.Osm/1.73 m²</i>									
1	5 to -38	9.04	5.587	618	568	92	348		56
2	26 to 46	7.79	5.134	659	605	92	354		54
<i>52 to 56 Glucose, I. V., 476 m.Osm/1.73 m²</i>									
3	58 to 79	18.5	9.768	528	505	96	314		59
4	79 to 100	12.5	7.438	595	534	90	325		55
<i>L.R. 14 yr.; 38.0 kg.; S.A. 1.30 m²; Loading Substance—Na₂SO₄ (I. V.)</i>									
P-1	-78 to -48	0.41	0.556	1356	1094	81			
P-2	-48 to -18	0.36	0.450	1251	903	72	[Na]	[SO ₄]	
<i>0 to 6 Na₂SO₄, 758 m.Osm/1.73 m²</i>									
N-1	-18 to 19	9.50	5.596	589	634	93	288	138	72
1	19 to 47	8.94	6.079	680	664	98	355	196	81
2	47 to 66	5.31	4.136	779	729	94	393	176	73
<i>71 to 78 Na₂SO₄, 506 m.Osm/1.73 m²</i>									
3	81 to 107	10.6	7.028	663	656	99	368	195	85
4	107 to 127	7.45	5.751	772	711	92	395	187	75
<i>G.P. 13 1/2 yr.; 42.5 kg.; S.A. 1.34 m²; Loading Substance—Creatinine (oral and I.V.)</i>									
P-1	-101 to -66	0.66	0.679	1029	840	82			
<i>-51 to -52 Creatinine, Oral, 160 m.Osm/1.73 m²</i>									
1	-66 to -36	0.83	0.827	996	825	83	5		1
2	-36 to -6	1.39	1.351	972	748	77	64		7
<i>0 to 4 Creatinine, I.V., 144 m.Osm/1.73 m²</i>									
3	5 to 25	6.97	4.607	661	589	89	261		40
4	25 to 46	3.51	2.664	754	676	90	276		37
<i>48 to 56 Creatinine, I.V., 218 m.Osm/1.73 m²</i>									
5	58 to 79	7.96	5.063	636	518	81	273		43
6	79 to 100	4.97	3.544	713	606	85	316		44

bution of the loading solute, and the solute load, i.e., the product of volume and concentration. The data on the individual solutes other than the loading solute and on the plasma levels of all solutes will be presented in the paper dealing with the renal osmotic work. It may be seen from an inspection of the table that the determined solutes as a rule accounted for 85 per cent \pm 10 per cent of the total osmolarity in the preloading specimens and for as much as 95 per cent during diuresis. The loading solute (or solutes in the case of salts) usually made up 60 \pm 10 per cent of the total solutes.

In figure 1 is presented for 111 periods a plot of the values of total osmolarity versus urinary flow. It is evident that with increasing flows there was a definite tendency to decreased urinary concentrations, with the rate of fall decreasing with increasing flows. These data over an extended range of flows confirm the observations of McCance *et al.* (6). The plot of the urine concentration versus urinary load is closely similar in form to that of the concentration versus flow and is therefore omitted from the presentation.

In figure 2, values for urinary flow are plotted against those of load. It may be seen that the data group themselves in a regular manner, regardless of the predominant solute in the urine over the whole range of flows observed, from 0.5 to 24 ml/min/1.73 m².

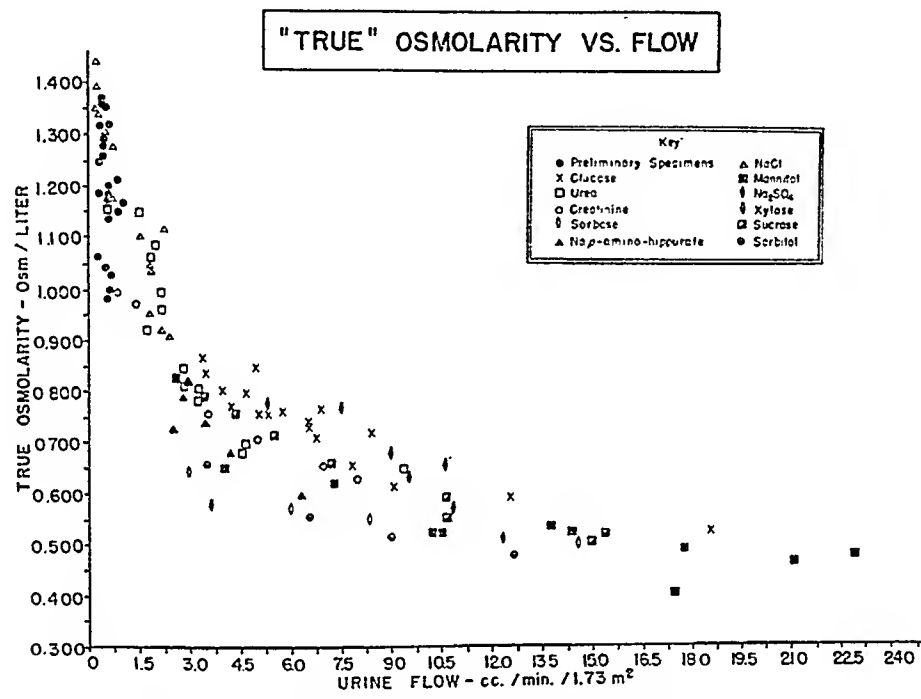


Fig. 1. URINE OSMOLARITY VERSUS FLOW. Data on 111 periods.

The dependence of urinary flow and osmolarity on load led to a consideration of the form of this relation. The assumption that with increasing loads the urinary concentration would tend to approach the plasma concentration in an asymptotic manner and that the decrease in concentration with respect to load is proportional to the concentration may be expressed in differential form as

$$(1) \quad - \frac{d \text{ conc.}}{d \text{ load}} = k (\text{conc.} - B)$$

where k is a proportionality constant and B is the total plasma osmolarity which in the following discussion is assumed to be 0.33 osmols per liter.³ Integration leads to the expression

$$(2) \quad - \ln(\text{conc.} - B) = k \text{ load} + A$$

³ In these experiments 0.33 was the approximate mean value of the calculated osmolarity of plasma during osmotic diuresis, with the extremes falling within ± 0.035 m.Osm/l. For the present basic derivations, variations of the plasma osmolarity within these limits are of no importance.

where A is the integration constant and the other symbols have the same meaning as before. In exponential form and rearranged the equation is

$$(3) \quad \text{conc.} = A' e^{-k \text{ load}} + B$$

Values for the constants A and k may be derived readily from equation 2, which is that of a straight line, with A , that is \ln of A' , as the intercept and k as the slope. The validity of the basic assumption may be tested by the rectilinearity of the plot of $\ln (\text{conc.} - B)$ versus load.

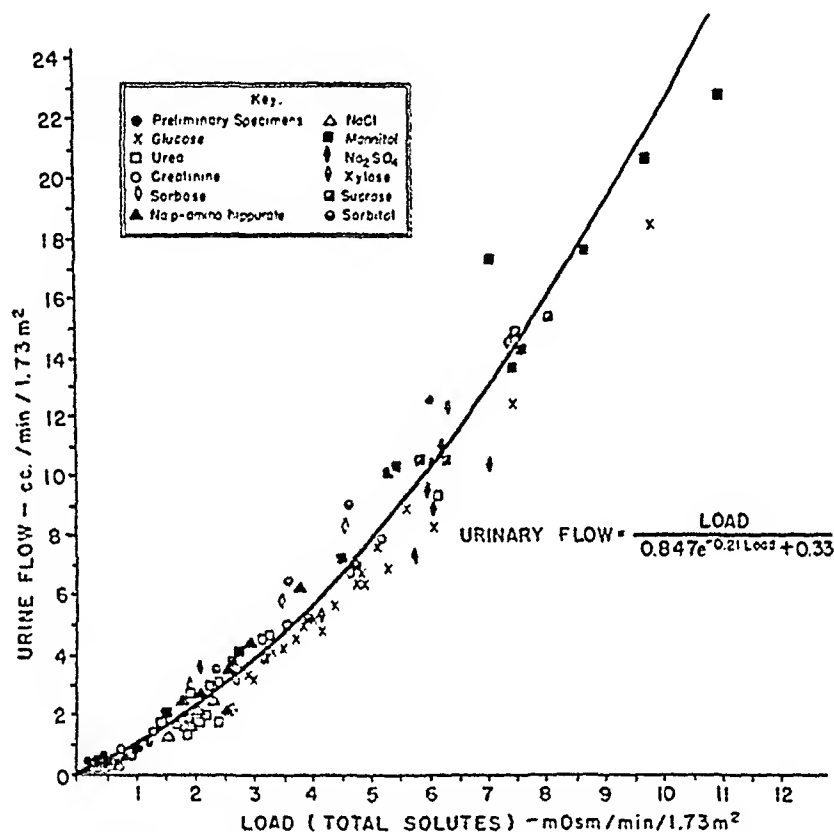


Fig. 2. URINE FLOW VERSUS LOAD. The line represents the values calculated from equation 5.

In figure 3 is presented the plot of the logarithms of the values for urinary concentration -0.33 against load. Also drawn is the straight line fitting the data, calculated by the least squares method. The equation of the line is $-\log (\text{conc.} - 0.33) = 0.091 \text{ load} - 0.072$. It may be seen that most data group themselves in a satisfactory manner around the straight line. Transformed to the exponential form with the base e the equation for urinary concentration may be written

$$(4) \quad \text{conc.} = 0.847 e^{-0.21 \text{ load}} + 0.33$$

where urinary concentration is expressed in osmols per liter.

From the identity of $\text{load} = \text{flow} \times \text{concentration}$, an equation for flow in terms of load may be obtained.

$$(5) \quad \text{urinary flow} = \frac{\text{load}}{A' e^{-k \text{ load}} + B}$$

The line in figure 2, containing the plot of flow versus load, was drawn on the basis of this equation. It is evident that it fits the data in a very satisfactory manner.

In figure 4 the flow/load values, which have the dimensions of the reciprocal of concentration, i.e. dilution, are plotted against load. The data are graphed on a

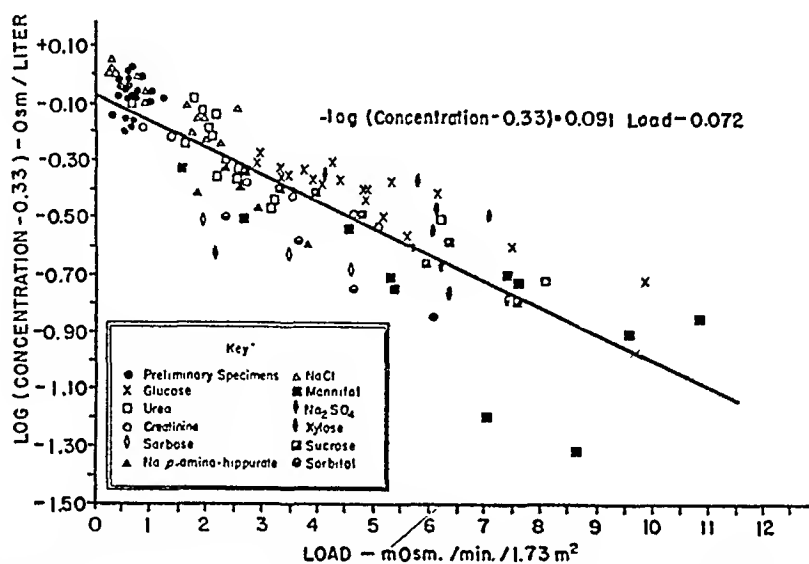


Fig. 3. PLOT OF LOG (URINE OSMOLARITY - 0.33) VERSUS LOAD. The line drawn through the points was calculated by the method of least squares.

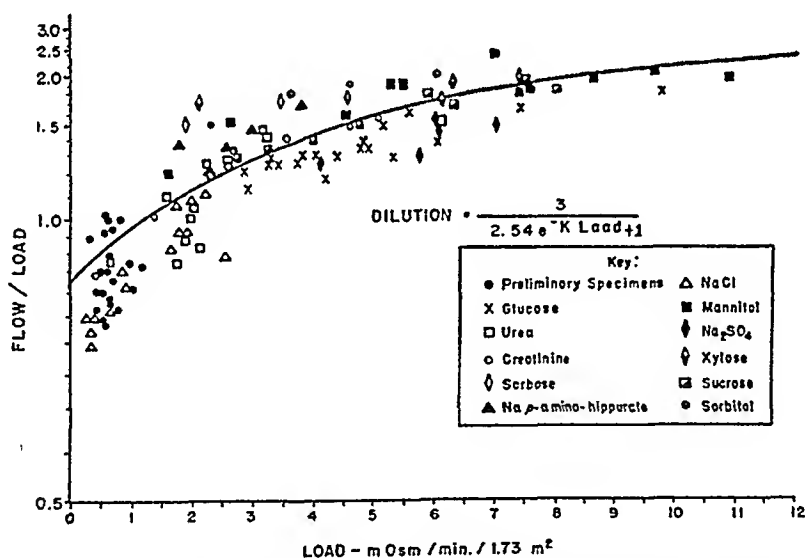


Fig. 4. URINE DILUTION (FLOW/LOAD) VERSUS LOAD. The ordinate, of the dimensions of a reciprocal of concentration, is plotted on a hyperbolic scale. The line was calculated from equation 6.

hyperbolic coordinate system for purposes of equal spacing. This graph makes most evident the asymptotic approach of dilution to the value of plasma with increasing load. The curve in the graph is based on the equation

$$(6) \quad \text{dilution} = \frac{3}{2.54e^{-0.21 \text{ load}} + 1}$$

which is the reciprocal of *equation 4*, with both numerator and denominator multiplied by 3. The equation is that of a logistic curve, with a lower asymptote of 0 and an upper asymptote of 3, i.e. the plasma dilution.

DISCUSSION

The dependence of urine flow on load, demonstrated clearly by figure 2, has interesting implications. A consideration of the variety of loading solutes employed indicates that the pattern of urine flow is largely independent of the ionic or chemical properties of the solutes. It is also independent of the mechanism of excretion, whether it be primarily by glomerular filtration (mannitol, sucrose, xylose, sulfate), filtration combined with tubular secretion (para-aminohippurate) or filtration combined with tubular reabsorption (urea, sodium chloride, glucose). A necessary conclusion is also that it is not the quantity of solutes in the glomerular filtrate, but only the amount finding its way into the urine that determines urinary flow. Consequently the urinary flow is not directly dependent on the level of the clearance or the plasma level of any solute. The plasma level and the extent of tubular secretion or absorption affect urinary flow only insofar as they determine the solute load.

The uniformity of the pattern clearly suggests the existence of a common denominator which is dependent on urinary load. Such a denominator is apparent when the process of diuresis is considered from the standpoint of water reabsorption. Then it is understandable that only the number and not the character of solutes determines the extent of water reabsorption, and thus the osmolarity of urine. Available knowledge on solute and water reabsorption in the tubules has led to the theory of two spatially separate phases of water reabsorption (21). With this in mind, the present data lead to the following interpretation: In the proximal portion of the tubule, active reabsorption of solutes, chiefly electrolytes, takes place in a nearly isotonic solution, with water passively accompanying the solutes, until only the solutes destined for excretion, the solute load, remain. From that point on a second phase of water reabsorption against a rising osmotic gradient, to produce a hyper-tonic urine, commences. For this second process two limiting conditions may be fixed from the onset: 1) that the solute concentration during osmotic diuresis cannot fall below the plasma level; and 2) that with decreasing loads and increasing time of passage through the distal tubule the concentration rises to some definite maximum value, which is the maximum osmotic potential attainable between plasma and urine. The derivations given in the preceding section are based on these two considerations.

The preceding assumptions and the mathematical derivations may be expressed equally well in terms of water reabsorption. The amount of water reabsorbed would then depend in an inverse manner on load, decreasing from a maximum value of 99.5 to 99.7 per cent to zero. According to this theory no definite percentage of water reabsorption can be assigned to the proximal portion of the tubules. The amount of water reabsorbed would depend in both proximal and distal tubular portions, even though in differing manner, on the solute load. Mathematically expressed:

$$(7) \quad \text{Total water reabsorbed} = R_p + R_d = \left(GFR - \frac{\text{load}}{B} \right) \div \left(\frac{\text{load}}{B} - \frac{\text{load}}{A'e^{-1 \text{ load}} \div B} \right)$$

where R_{pr} and R_d are the amounts of water reabsorbed in the proximal and distal portions of the tubules respectively, B is the total osmolarity of the plasma and GFR is the volume of the glomerular filtrate. The first two terms of the equation define the water reabsorption in the proximal tubule and the last two that in the distal tubule. One consequence of these derivations is the absence of a minimum of water reabsorption. Data on osmotic diuresis in animals (22), where urine flows as high as 60 per cent of the glomerular filtration rate were measured, would lend support to this conclusion.

Although a detailed discussion of renal osmotic work will be deferred to a later publication, a few comments are here in order. In both proximal and distal tubules renal osmotic work is performed. In the proximal tubule, although the total osmolarity is assumed to remain constant at the plasma level, changes in concentration of the individual solutes from that existing in the glomerular filtrate take place. In the distal portion of the tubules, the percentage composition of solutes remains constant, since no further absorption of solutes is assumed to occur, but reabsorption of water against a rising osmotic gradient proceeds.

Obviously the question arises as to the bearing of these experiments on the rôle of the antidiuretic hormone in osmotic diuresis. The constancy of the pattern may be interpreted as indicative of the constancy of the response elicited, which then would enter as one of many other specific factors into the proportionality constant relating load to the diuretic response. In the absence of the hormone, then, the relationship of flow to load should be expressible by an equation similar to that here derived, but differing in the magnitude of the proportionality constant.

The constant relationship between urinary flow and load by no means implies a proportional diuretic response to the administration of equal amounts of different solutes. Here the factors of metabolic removal, of distribution volume, and of the manner of renal excretion, as they affect the urinary load have to be considered. For a substance excreted at the level of glomerular filtration, the relation that $P \times GFR = \text{load}$ implies a direct proportionality of load to the plasma level. If the clearance is a definite fraction of the filtration rate a similar constant relationship will obtain. Any reabsorption or secretion process, if operative will affect the solute load in a specific manner. The volume of distribution enters into the considerations as it affects the plasma level. The greater the volume the lower the plasma level, and consequently the load, for a given amount of solute administered. Finally, metabolic disappearance if occurring will negatively affect the renal load. On the basis of these considerations a scale of diuretic efficiency of the various solutes may be established. The most effective diuretic solute would distribute itself in the smallest volume, would not be metabolized and would be excreted at the level of glomerular filtration, perhaps even boosted by tubular secretion. In this study, the most effective substances were mannitol and sucrose. Sodium sulfate ranked next insofar as the sulfate ion was concerned. On the opposite end of the scale were found urea and sodium chloride, urea both because of its large volume of distribution and a moderate tubular reabsorption and sodium chloride primarily because of its extensive tubular reabsorption.

SUMMARY

The pattern of concentration and flow of urine during osmotic diuresis in dehydrated man was studied. Eleven loading solutes, glucose, sucrose, mannitol, sorbitol, sorbose, xylose, urea, creatinine, sodium para-aminohippurate, sodium sulfate, and sodium chloride, were employed. A definite relationship between urinary flow of concentration and load was found independent of the ionic or chemical character of the solutes, or of the mode of their renal excretion. The assumption that the rate of decrease of the urinary concentration is proportional to the concentration and that the urinary concentration cannot fall below that of plasma, leads to an exponential equation: $\text{Concentration} = A'e^{-k \text{ load}} + B$, where A' is a constant defining the urinary concentration at zero load, B is the total osmolarity of plasma, and k is a proportionality factor. A related expression may be derived for urinary flow.

On the basis of these data and on the assumption of an isotonic absorption of solutes and water in the proximal tubule the following equation for water reabsorption in the nephron is proposed: $\text{Total water reabsorbed} = R_{pr} + R_d = \left(\text{GFR} - \frac{\text{load}}{B} \right) + \left(\frac{\text{load}}{B} - \frac{\text{load}}{A'e^{-k \text{ load}} + B} \right)$ where R_{pr} and R_d are the amounts of water reabsorbed in the proximal and distal portions of the tubules respectively, B is the total osmolarity of the plasma and GFR is the glomerular filtration rate. The first two terms of the equation define the water reabsorption in the proximal tubule and the last two urine flow.

The diuretic effect of a loading solute may be assessed on the basis of its volume of distribution, the manner of its renal excretion, and its metabolic fate, factors affecting the solute load.

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IMPROVEMENTS ON THE ARTIFICIAL KIDNEY: AN EXPERIMENTAL STUDY OF ITS APPLICATION TO DOGS BILATERALLY NEPHRECTOMIZED OR OTHERWISE DEPRIVED OF RENAL FUNCTION¹

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THE utilization of a semi-permeable membrane as an 'artificial kidney' has been attempted repeatedly by investigators, since it was first realized that the removal of waste products by the kidney was in large part a process of ultrafiltration through the glomerulus. The successful operation of such an artificial kidney would permit not only experimental studies on the bilaterally nephrectomized animal or one otherwise rendered devoid of renal function but might also have clinical application to the human patient whose renal excretory function is temporarily in abeyance. The earliest attempts at utilizing an artificial kidney, such as those of Abel and his collaborators (1), were futile because of the difficulty of preparing an adequate dialyzing membrane and the unavailability of a non-toxic anticoagulant. With the availability commercially of artificial membranes readily permeable to urea and other diffusible substances and of heparin as a non-toxic and naturally occurring anticoagulant, the concept of utilizing an artificial kidney has again come to the fore. The recent reports of Kolff (2, 3), Murray *et al.* (4, 5), and Alwall *et al.* (6-8) in particular have attracted considerable attention.

Although several reports have appeared in the scientific and lay press concerning the value of an artificial kidney, an analysis of the available literature leaves doubt as to whether or not the life of the animals or patients has actually been prolonged by the application of this procedure. Insofar as the use of the artificial kidney on the human patient is concerned, it is difficult to judge from the available data as to the value of the procedure since recovery follows the use of more conservative measures in the majority of cases (9). It is essential, therefore, to rely upon animal experiments to demonstrate the effectiveness and value of the procedure.

Kolff (3), Lam and Ponka (10), and MacLean *et al.* (11) have applied the procedure to patients without any preliminary trial on animals. Murray *et al.* (4, 5) implanted the ureter into the small bowel in a dog but present no data on the survival of this animal. Alwall (7) kept a rabbit with ureters ligated alive for only 5½ days and another with acute mercurial poisoning for 6 days. Skeggs and Leonards (12) were also unable to keep dogs alive for a significantly longer period than their controls by the use of their artificial kidney. There is thus no available proof as to the efficacy of an artificial kidney in prolonging the life of either laboratory animals or patients and its application clinically has been premature. We, therefore, undertook a study to determine the sources of difficulty in the available procedures and how these might be overcome.

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MATERIALS AND METHODS

The present paper is based on a study of 110 dogs deprived of renal excretory function by 1) bilateral nephrectomy, 2) unilateral ligation and division of one ureter with removal of the contralateral kidney, 3) bilateral ligation and division of both ureters, 4) implantation of one ureter into the small bowel with removal of the contralateral kidney or 5) implantation of one ureter into the vena cava with removal of the contralateral kidney. Following these operations, they were maintained on an electrolyte-free diet of casein², glucose and lard. Animals maintained on such a diet remain in good health and do not show the rapidly downhill course usually observed and attributed erroneously to the effects of uremia. They survive untreated usually for periods of 5 to 7 days.

Four to 6 days following the above-mentioned operations, cannulae were inserted into the femoral artery and vein under procaine local anesthesia with aseptic surgical technique and the animal was connected to the artificial kidney. This was a replica of that described by Kolff (2) except for the drum which was made of perforated stainless steel instead of wooden laths as in Kolff's apparatus.

Plasma sodiums were determined by a modification of the Butler-Tuthill (13) method; plasma chlorides, by the colorimetric method of Stiff (14); plasma carbon-dioxide combining power, by the direct method of Van Slyke and Cullen (15); plasma potassium, by the photoelectric method of Looney and Dyer (16); serum calcium, by the Clark-Collip (17) modification of the Kramer-Tisdall method; serum magnesium by Hawk, Oser and Summerson's (18) modification of Denis' method; plasma specific gravities, by the technique of Phillips, Van Slyke *et al.* as described by Hawk, Oser and Summerson (18); plasma urea, by Summerson's modification (18) of the Van Slyke-Cullen procedure; plasma non-protein nitrogen and creatinine, by the colorimetric methods of Folin and Wu (19); inorganic phosphates by the colorimetric method of Fiske and Subbarow (20); inorganic sulfates, by the colorimetric method of Letonoff and Reinhold (21); and free and combined phenols, by the method of Theis and Benedict (22). The colorimetric methods used were adapted for a Coleman Universal Spectrophotometer or Leitz colorimeter.

EXPERIMENTAL

Our first attempts to apply the artificial kidney met invariably with failure. Not only was it impossible to prolong the life of the bilaterally nephrectomized animal, but its application actually led to their earlier demise as compared to untreated control animals (9). Even normal animals subjected to the apparatus for 2 to 3 hours succumbed within a few days.

The failure of the technique as suggested by Kolff (2) was due, we found, to a number of fundamental defects. The following, in particular, were serious deterrents to its successful application: 1) the composition of the bath not only failed to maintain adequately the normality of the chemical composition of the blood, but in addition caused a marked degree of hemolysis; 2) the membrane reacted with the blood to cause hemolysis and other toxic effects, despite thorough preliminary washings with water; 3) the pump on the original apparatus of Kolff caused a severe degree of hemolysis; 4) the dosage of heparin had to be adjusted accurately to avoid undue effects and prevent coagulation, and antiheparin substances were needed to prevent hemorrhage from the wound after the procedure; and 5) variations in the blood con-

² The casein was supplied by Mead-Johnson Company through the courtesy of Dr. Charles E. Bills.

tent of the apparatus resulted in depletion or overload of the vascular system of the experimental animal with subsequent death.

Composition of Dialyzing Fluid

The composition of the dialyzing fluid used in removing the waste products from the body is of greatest importance since it determines the final composition of the blood and body fluids with which it enters into equilibrium. Previous authors have suggested fluids of various composition, none of which, however, have been found by us to give optimal results. Our aim has been to use a solution of such composition as

TABLE 1. COMPOSITION OF DIALYZING BATH USED IN ARTIFICIAL KIDNEY

SOLUTE	CONTENT	Na ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	CO ₃ ⁻⁻ HCO ₃ ⁻
	gm./l.	mEq. per liter				
NaCl.....	5.77	98.7	3.6	1.1	98.7	11.0 29.8
CaCl ₂	0.20				3.6	
MgCl ₂	0.05				1.1	
Na ₂ CO ₃	0.58	11.0				
NaHCO ₃	2.50	29.8				
Glucose.....	2.00					
Total.....		139.5	3.6	1.1	103.4	40.8

TABLE 2. TYPICAL EFFECTS OF A 3-HOUR PERIOD OF DIALYSIS INTO THE ARTIFICIAL KIDNEY ON THE SODIUM, CHLORIDE, AND CARBON DIOXIDE COMBINING POWER OF BLOOD PLASMA OF DOGS DEPRIVED OF RENAL EXCRETORY FUNCTION

SODIUM		CHLORIDE		CARBON DIOXIDE COMBINING POWER	
Before	After	Before	After	Before	After
mEq. per liter					
157	150	111	98	22	32
165	152	96	95	28	30
153	148	110	104	23	33
140	150	110	107	16	27

would convert the plasma to as near normal a state as possible. The composition of the blood plasma prior and subsequent to the dialysis was used to arrive empirically at a composition which gave optimal results. The composition of this solution, the total volume of which was 120 liters, is given in table 1. It differs in several notable respects from the solutions recommended by previous workers for use with either the artificial kidney (2, 5, 6) or for peritoneal lavage (23-25). In table 2 are reproduced values for the sodium, chloride and carbon-dioxide combining power of the blood plasma before and after dialysis. It will be noted that the procedure was effective in correcting the decreased carbon-dioxide combining power that is associated with uremia and adjusting abnormalities in the sodium and chloride contents of the plasma.

In order to adjust promptly the pH of the bath, both sodium carbonate and sodium bicarbonate were used, and their proper ratio was maintained by bubbling a mixture of carbon dioxide and oxygen through the bath.

Data for potassium serum levels are given in table 3. Although potassium could be kept at the desired normal level by adding it to the bath, it was found empirically that its addition was unnecessary. The tissue breakdown, plus the hemolysis produced by the procedure, was sufficient to rectify the potassium level of the blood plasma following its depletion by the dialysis. Thus the blood plasma potassium of *dog 1* of table 3 at the end of a previous dialysis was 0.1 mEq/liter but had risen to 2.7 when the animal was subjected to a subsequent dialysis.

TABLE 3. POTASSIUM CONTENT OF ARTERIAL BLOOD PLASMA OF 2 DOGS BEFORE AND AFTER APPLICATION OF THE ARTIFICIAL KIDNEY FOR A PERIOD OF 3 HOURS¹

Dog No.	¹ mEq. per liter ²	
Before dialysis.....	2.7	4.2
After dialysis.....	0.1	3.8

¹ In *dog 1*, one ureter had been implanted into the small bowel and the contralateral kidney removed 21 days prior to the dialysis; in *dog 2*, bilateral nephrectomy had been performed 5 days previously. The dialyzing bath in the experiment on *dog 1* contained no potassium; it contained 3.0 mEq./l. in the experiment on *dog 2*.

TABLE 4. CALCIUM AND MAGNESIUM CONTENT OF ARTERIAL BLOOD SERUM OF A DOG MAINTAINED FOR 30 DAYS FOLLOWING IMPLANTATION OF THE RIGHT URETER INTO THE SMALL INTESTINE AND REMOVAL OF THE CONTRALATERAL KIDNEY

DAYS FOLLOWING NEPHRECTOMY	CALCIUM		MAGNESIUM	
	Before dialysis	After dialysis	Before dialysis	After dialysis
	mEq. per liter			
9	5.0		1.3	
13		5.1	1.4	1.4
17	3.8	3.8	1.4	1.3
25	3.9	5.1	1.3	1.6
30	5.1		2.2	

Kolff (2) did not add calcium to the dialyzing fluid but its omission results in the development of cardiac arrhythmias which are relieved by the administration of calcium chloride intravenously. When calcium and magnesium chlorides are added to the bath after the addition of the other salts and adjustment of the pH to 7.4, as described below, no precipitation results. Typical serum calcium and magnesium values are given in table 4.

The marked hemolysis observed following the use of Kolff's procedure was found to be due in part to the alkalinity of his dialyzing solution. When this was reduced by bubbling carbon-dioxide through the bath, hemolysis was prevented but depletion of the red cell of its oxygen resulted in a shift of large amounts of chloride into the cell. A mixture of oxygen and carbon dioxide was, therefore, bubbled through the bath, the pH of which was maintained at 7.39 ± 0.01 by adjusting the ratio of the two gases by

means of flow meters. The pH was determined continuously by a Beckman pH meter with an extension electrode in the bath. Efficient bubbling of the mixture of oxygen and carbon dioxide was produced by passing the gases (7-12 liters of oxygen, and 1.2 to 2.6 liters of carbon dioxide per minute) through a submerged, weighted cloth tube 75 cm. long and 2 cm. in diameter. To maintain a constant pH on the exposed as well as on the submerged part of the drum, a hood of transparent plastic 'cloth' was placed over the apparatus. The temperature of the bath was maintained at $37.5^{\circ} \pm 0.5^{\circ} C$.

Dialyzing Membrane

Approximately 38 meters of Visking 'No Jax' sausage casing 1.8 cm. in diameter was used as the dialyzing membrane. Such membranes cause marked hemolysis even at room temperature and after washing with water for as long as 96 hours. When used for dialysis of the blood of the experimental animal, such membranes induce reactions with vomiting, shivering without an elevation in rectal temperature, a fall of blood pressure etc. Similar effects were obtained when other membranes were tested. In attempting to modify its hemolytic and toxic actions, the membrane was treated with various oxidizing and reducing agents, acids and alkalies, surface acting materials, and organic solvents. In general, it was found that treatment with oxidizing agents and alkaline solutions increased the hemolytic effect, while reducing agents, organic acids and polar solvents decreased it. This hemolytic action of the commonly available dialyzing membranes is apparently due to the fact that they are not inert but possess chemically active groups which react with the blood.

The final procedure adopted for treating the membrane in order to reduce hemolysis and toxicity to a negligible minimum was as follows. The membrane was first washed with tap water for 1 to 2 hours in order to remove glycerine and other water-soluble contaminants. It was then placed in 4 liters of 10 per cent formaldehyde for at least 12 hours and again washed with tap water for 10 to 12 hours. The formaldehyde, being an active reducing agent, removes oxidizing groups from the membrane which are largely responsible for the hemolytic action of cellophane. The formaldehyde and its reaction products were then removed by placing the membrane in 3 liters of chemically pure dioxane (freshly prepared by distillation from metallic sodium) for 12 to 18 hours. After washing with tap water for 8 to 10 hours to remove the dioxane, the entire process of treatment with formaldehyde and dioxane was repeated using fresh reagents.

The membrane treated as just described was washed immediately before using for 30 to 45 minutes with at least 60 liters of fresh, triple distilled water and tested for leaks by filling with water under pressure. The membrane was finally rinsed with 500 cc. of sterile pyrogen-free isotonic saline. This, as well as the previous washings, was carried out with the membrane on the rotating drum of the apparatus with water running through the lumen as well as dripping over its surface.

Leaks in the membrane were avoided by care in handling the dry, collapsed tubing. To aid in the detection of such leaks developing during the dialysis, a white gauze cloth was suspended from a metal bar extending across the length of the drum.

The Pump

Since the force exerted by 'Archimedes screw' will not deliver the blood against a column higher than 17.5 cm. below the axle of the drum, Kolff (2) used a mechanical pump to raise the blood above the level of the patient and allowed it to return to the vein by the force of gravity. We found that the blood circulating through the pump for 5 minutes underwent marked hemolysis. We, therefore, eliminated the pump by placing the animal well below the axle of the drum. The arterial pressure sufficed to raise the blood to the machine, and the 'Archimedes screw' then propelled the blood into a reservoir placed below the axle of the drum, from which it returned to the vein of the animal by the force of gravity.

Anticoagulants and Coagulants

The optimum initial heparin dosage was found to be 3.3 mg/kg. of body weight. Ten milligrams of this dose was used to prevent coagulation of the donor blood used to fill the membrane. The remainder was given intravenously before starting the dialysis. A maintenance dose of 0.33 mg/kg. of body weight was administered every 30 minutes except for the final half hour before the end of the dialysis. Although this dosage was at first determined empirically, subsequent studies by heparin titration demonstrated that it was satisfactory. For more exact dosage, titration of the circulating blood for its heparin content is desirable and should be carried out when applying the procedure to the human subject. The use of an inadequate dose of heparin results in clotting and embolus formation, while an excess may result in fatal hemorrhage, or possibly in massive platelet embolism (26). To neutralize the excess heparin present in the body, toluidine blue (2 mg/kg. of body weight in 1 % solution)³ was administered intravenously at the end of the dialysis. In this way, hemorrhage at the site of cannulation of the vessels was avoided.

Adjustment of Circulatory Volume

Because of its distensibility, the volume of blood in the dialyzing membrane varies with the rate of blood flow. To avoid these fluctuations, the animal was placed on a scale sensitive to 10 grams and balanced prior to the dialysis. By manipulating the level of blood in the Salvarsan flask used as a reservoir, the return of the blood to the experimental animal was controlled so that its weight was maintained constant. In this way, overloading or depletion of the circulation with its attendant effects was avoided. Attention to the weight of the animal also allowed detection of any sudden changes in either the inflow or outflow of blood due to clotting in the machine or to kinking of the cannulae. However, a gradual gain in weight may occur as a result of absorption of water if the animal is dehydrated. For this reason, if the flow remains constant, the animal is allowed to gain weight during the dialysis. This gain in weight may amount to 500 grams or more. That it is not due to hemodilution is evidenced by the fact that the specific gravity of the blood serum remained constant or varied only slightly (± 0.001) throughout the procedure even when water was absorbed from the bath.

³ Ampules of toluidine blue were supplied by the Lakeside Laboratories through the courtesy of Dr. H. L. Daiell.

Other Technical Details

Instead of the glass beads suggested by Kolff (2), a gauze filter was placed in the system between the reservoir and the venous cannula to remove any blood clots. The donor's blood used to fill the membrane was also filtered through a cloth filter impregnated with bakelite (27).

The cellophane was attached to the rubber connections at the end of the apparatus by surgical silk and to the drum by surgical gauze. Gauze knots were also placed through the metal perforations in a row along the length of the drum in order to prevent the cellophane from slipping. Ordinary cotton string is unsuitable for this purpose since it contains sizing which dissolves in the bath and causes reactions in the animal.

Venous and arterial manometers were attached by T-tubes so that determinations of these pressures could be made throughout the procedure. Latex surgical tubing was used throughout in making connections from the cannulae to the machine. All the glass, metal and rubber connections were thoroughly rinsed with tap water following each experiment, soaked in 5 per cent hydrogen peroxide and thoroughly rinsed with distilled water and sterilized.

Blood for filling the membrane was collected from a donor that had been cross-matched with the experimental animal. After infiltration with 2 per cent procaine-epinephrine, the femoral artery was cannulated and the animal was allowed to exsanguinate itself into a paraffin-coated flask containing 10 mg. of heparin.

When necessary, fresh concentrated red cells were transfused into the dog at the conclusion of the dialysis in an amount indicated by the hematocrit and the degree of hemolysis produced by the procedure. At this time also 200,000 U of crystalline penicillin G was administered intravenously. To replace the loss of water-soluble vitamins, these were also either administered intravenously or preferably added to the animal's food.

RESULTS

Removal of Catabolites

Urea and other nitrogenous waste products. In table 5 are recorded data on the rate of removal of urea, creatinine and non-protein-nitrogen from the blood of four animals. The plasma urea and non-protein nitrogen levels of table 5, expressed in percentage of their initial levels, are in all cases comparable, indicating that most, if not all, of the non-protein-nitrogenous constituents are being removed at comparable rates. Although creatinine also, as shown in the table, diffuses readily across the membranes, a dialysis of three hours is not always sufficient to reduce the creatinine level to normal. Urea diffuses most readily through the membrane despite the fact that it is present in the blood, chiefly in the form of the dimer, $(\text{CONH}_2)_2$ (28).

Inorganic sulfates. In table 6 are reproduced the analytical data for inorganic sulfates on 8 dialyses on 2 animals. It will be noted that the sulfate levels were not reduced to normal by the dialysis. Nevertheless, this ion is removed fairly efficiently and the levels maintained were sufficient to permit survivals to the 17th and 20th days in these animals.

TABLE 5. BLOOD PLASMA UREA, NON-PROTEIN-NITROGEN AND CREATININE BEFORE AND AFTER 8 DIALYSES ON 4 DOGS¹

DOG	DAYS FOLLOWING OPERATION	BEFORE DIALYSIS			AFTER DIALYSIS		
		Urea	NPN	Creatinine	Urea	NPN	Creatinine
		<i>mg/100 cc. plasma</i>					
1	4	344	445	3.1	68	46	2.3
	7	455		3.0	47	30	2.0
2	5	308	450	3.0	76	48	2.5
	8	348	200	3.1	178	107	2.4
3	5	396	180	3.1	98	48	2.2
	8	225	133	2.9	81	55	2.1
4	6	360	278	3.0	141	124	1.7
	9	330	230	4.0	111	71	1.9

¹ In dog 1, both ureters were ligated and divided; in dogs 2, 3, and 4, both kidneys were removed.

TABLE 6. INORGANIC SULFATE CONTENT OF THE ARTERIAL BLOOD PLASMA OF 2 DOGS BEFORE AND AFTER 3 HOURS OF DIALYSIS WITH THE ARTIFICIAL KIDNEY¹

DOG	DAYS FOLLOWING OPERATION	INORGANIC SULFATE CONTENT OF BLOOD		DOG	DAYS FOLLOWING OPERATION	INORGANIC SULFATE CONTENT OF BLOOD	
		Before dialysis	After dialysis			Before dialysis	After dialysis
		<i>mEq. per liter</i>				<i>mEq. per liter</i>	
1	9	4.6	2.3	2	7	2.7	0.6
	11	5.0	2.3		9	4.2	1.8
	14	5.0	1.7		11	5.0	0.8
					14	4.8	0.4

¹ Dog 1 was bilaterally nephrectomized; in dog 2, one ureter was implanted into the small bowel and the contralateral kidney removed.

TABLE 7. PHOSPHATE CONTENT OF ARTERIAL BLOOD PLASMA OF 3 DOGS BEFORE AND AFTER DIALYSIS WITH THE ARTIFICIAL KIDNEY¹

EXPERIMENT NO.	BEFORE DIALYSIS <i>mEq. per liter</i>	AFTER DIALYSIS <i>mEq. per liter</i>	EXPERIMENT NO.	BEFORE DIALYSIS <i>mEq. per liter</i>	AFTER DIALYSIS <i>mEq. per liter</i>
1	28.3	14.6	3	8.4	4.9
2	27.5	6.8	4	6.2	3.0

¹ Experiments 1 and 2 were performed 5 days following bilateral nephrectomy; experiments 3 and 4 were performed on the 13th and 17th days following unilateral nephrectomy with implantation of the ureter of the contralateral kidney into the small bowel.

TABLE 8. PHENOL CONTENT OF ARTERIAL BLOOD OF A DOG 8 DAYS FOLLOWING BILATERAL LIGATION OF THE URETERS, BEFORE AND AFTER 3 HOURS OF DIALYSIS ON THE ARTIFICIAL KIDNEY

	PHENOL CONTENT OF BLOOD		
	Total	Free <i>mg/100 cc.</i>	Conjugated
Before dialysis.....	1.37	1.15	0.22
After dialysis.....	0.80	0.75	0.05
After passage of blood through dialyzer.....	0.45	0.42	0.03

Inorganic phosphates. Table 7 demonstrates that although no phosphate is added to the bath, severe depletion of the inorganic phosphate is not produced and in dialyses of three hours or less, sufficient phosphate is dialyzed from the blood to maintain relatively normal blood levels.

Phenols. It has often been claimed that many of the symptoms observed in uremia are due to the accumulation of phenolic substances in the body. The blood of animals deprived of renal excretory function accumulates phenols and other substances (pyruvic acid, hydroquinones, catechols, aldehydes etc.) which react like phenols with diazotized para-nitroaniline. These substances, as shown in table 8, are dialyzable and are removed from the blood by the artificial kidney.

Rate of Dialysis

A comparison of the urea concentration of the blood before and after its passage through the dialyzer permits one to determine the optimal rate of blood flow to be maintained and the time necessary for attaining equilibrium between the body and the dialyzing bath. With a blood flow of 120 to 150 cc., the removal of urea from the blood is fairly complete and equilibrium is attained between the sodium, potassium, calcium, chloride and carbonate of the blood and the bath. This rate of blood flow was, therefore, maintained wherever possible.

The time necessary for removing the urea from the body by dialysis may be calculated by application of the 'compound-interest law' formula: $u_t = ue^{-\mu t}$, where u_t represents the amount of urea left after time t , u = the amount of urea present at the beginning of the experiment e = base of natural logarithms, 0.4343, and μ = the fraction of the total urea removed per minute.

Applying the above equation to a dog of average size (10 kg.) with a blood flow of 150 cc. per minute through the dialyzer for a period of 3 hours and an initial urea level of 500 mg/100 cc. of blood, $u_t = 0.5 \times 80 \times .4343^{-180(150)} = 2.42$. The urea content at the end of the experiment would be $\frac{2.42}{80} = 30$ mg/100 cc. In actual practice

the removal has not been as good as this due to the failure of the dialyzer to remove all of the urea and probably to a slight delay in attaining equilibrium between the blood and the tissues. To maintain a dog deprived of its renal excretory function in good condition, it was necessary to subject it to the procedure for a period of 3 hours on the 4th or 5th day after operation and on every 3d or 4th day thereafter.

In figure 1 are reproduced data on the concentration of the more important constituents of the blood plasma on a dog surviving for 19.5 days following bilateral nephrectomy. This animal was dialyzed for 2 to 3 hours on the 7th, 9th, 11th, 14th and 17th days following the removal of the second kidney. It is noted that the sodium, chloride and carbon dioxide combining powers of the blood are markedly reduced on the 5th day following nephrectomy. Inasmuch as this dog was not vomiting nor having diarrhea during this period, the reduction in sodium and chloride must indicate a passage of these ions from the extracellular to the intracellular fluid spaces. Also to be noted is the continued rise of the carbon dioxide combining power during the two days following the dialysis. Inasmuch as the concentrations of inorganic phosphate, sulfates and organic acids are increasing during this time, some

other factor must be involved in the production of acidosis in the uremic animal than merely the increase of acid catabolites. A further study of this phenomenon is indicated.

DISCUSSION

By adherence to the procedure as outlined above, it has been possible to maintain dogs deprived of all renal excretory function in good condition for periods beyond those ever encountered in untreated animals. Our control animals always succumb within 6 days, whereas those subjected to the artificial kidney may be kept alive for 8 to 30 or more days, depending on the manner in which they are deprived of their renal excretory function. Bilaterally nephrectomized dogs cannot be maintained

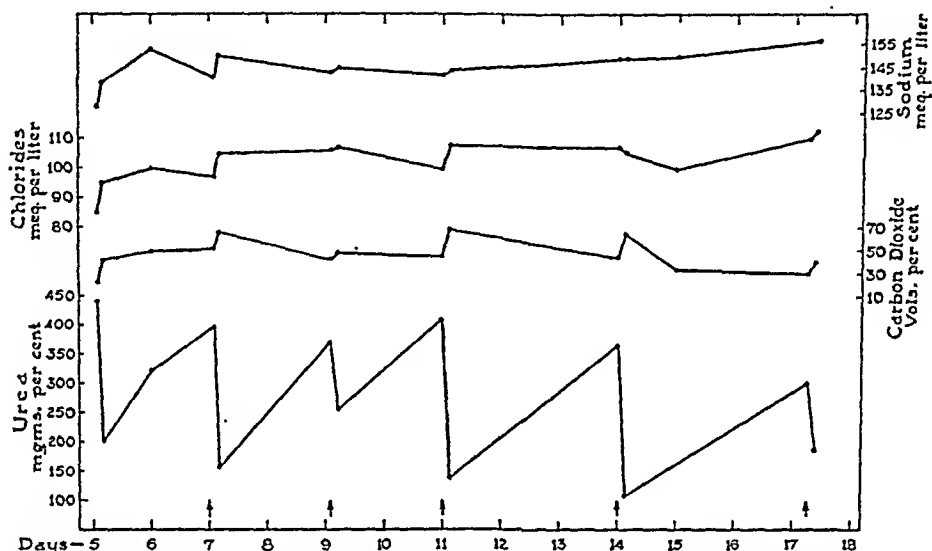


Fig. 1. SODIUM, CHLORIDE, UREA, AND CARBON DIOXIDE combining power of the blood plasma of a bilaterally nephrectomized dog surviving for 19.5 days following the second nephrectomy. Concentrations are plotted as ordinates against days following nephrectomy as abscissae. Arrows indicate the days on which dialysis for 2 to 3 hours was performed.

indefinitely by application of the artificial kidney, since these animals succumb after one to three weeks from malignant hypertension and at autopsy reveal the lesions of this disorder. On the other hand, when an intact kidney remains in the body, despite the fact that it is deprived of its excretory function, as when one ureter is implanted in the small bowel and the contralateral kidney removed, the animal can be maintained for prolonged periods (30 days or more). Such animals at autopsy do not reveal the characteristic lesions observed in the nephrectomized animals. The blood pressure changes in these groups of animals and their bearing on the problem of the pathogenesis of hypertension will be reported elsewhere (29).

It may be concluded from the present study that dialysis of the blood across a cellophane membrane removes adequately the waste products from the organism and that death in uremia is a result of the accumulation of such waste products. The results also prove the efficacy of the 'artificial kidney' in replacing known excretory function.

SUMMARY

The artificial kidney has been applied successfully to dogs deprived of renal excretory function by bilateral nephrectomy or other procedures. A study of the artificial kidney as used by previous workers revealed numerous sources of difficulty. Details are given for the correction of these defects including methods for the chemical treatment of the dialyzing membrane, the composition of the dialyzing fluid, the prevention of overload of the circulation, and other fundamental considerations which previous investigators have overlooked. By attention to these details it has been possible to maintain dogs deprived of renal excretory function for a period of a month when they have been killed for histologic study.

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BLOOD HYPERTENSINOGEN IN EARLY HEMORRHAGE HYPOTENSION

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COLLINS and Hamilton (1) reported that in hemorrhaged dogs there was often an early increase in renin substrate (hypertensinogen) which later decreased if the hypotension was of sufficient intensity and duration. Using guinea-pig ilium for assays, they demonstrated an increase in blood hypertensinogen at from 50 to 237 minutes after the start of bleeding for nephrectomized dogs and from 60 to 632 minutes in intact dogs with no inactivation of adrenaline. These authors point out that while almost half of the animals studied showed an early rise in hypertensinogen following hemorrhage, in the remainder, this change might have been missed by a failure to take samples at the proper time.

The minimal time required for the appearance of an increase in hypertensinogen noted by these writers was 50 minutes, but if the release of hypertensinogen following hemorrhage is to play any beneficial rôle during the critical lowering of blood pressure attendant on hemorrhage it would seem that an increase in hypertensinogen should appear much earlier than 50 minutes after the onset of the hemorrhage. Accordingly the experiments reported here were planned to obtain data on the earliest rise in hypertensinogen in the blood following hemorrhage.

PROCEDURE

Fifteen selected male dogs, 5.5 to 19.0 kg., were used in these experiments. These dogs were given intraperitoneally 32.5 mg. of sodium pentobarbital per kilogram of body weight, and the operative procedures were begun about 30 minutes after this injection. The retroperitoneal approach was used when nephrectomy was performed, the only loss of blood being a very small amount of superficial bleeding, and of course such blood as was actually in the kidneys. Blood pressure was recorded directly from the right common carotid artery. Hemorrhage when desired was produced by releasing blood from the cannulized left femoral artery. The dogs were bled quickly until a blood pressure level was reached at which the usual vasoconstriction could no longer raise blood pressure. This procedure required from 16 to 32 minutes and the actual pressure level of hypotension produced varied from 44 to 72 mm. Hg.

In the experimental test there were two groups of dogs, those with kidneys undisturbed and those with complete bilateral nephrectomy. In the first group 3 dogs were carried as controls without hemorrhage and 5 were subjected to quick severe

hemorrhage. In the nephrectomized group 2 dogs were not hemorrhaged and 5 were bled as described.

Hog renin standardized according to the method of Goldblatt, *et al.* (2) was used throughout these experiments as a test of the hypertensinogen content of the blood in the animal, by measuring the blood pressure response to a standard injection of renin. Routinely each dog was given one intravenous injection of 1.0 cc. of standardized hog renin about 10 minutes after the blood pressure stabilized following the operative procedures. Between 20 and 30 minutes following this first injection a second injection of 1.0 cc. of renin was given if the dog was not to be bled. If the dog was to be bled, the hemorrhage was begun about 20 minutes after the first injection, the second injection made immediately following the hemorrhage.

TABLE 1. BLOOD PRESSURE RESPONSES FOLLOWING INITIAL INJECTIONS OF 1.0 CC. HOG RENIN, NO HEMORRHAGE

DOG	KIDNEY CONDITION	BLOOD PRESSURE			DOG	KIDNEY CONDITION	BLOOD PRESSURE		
		Initial	Actual rise	Percent- age rise			Initial	Actual rise	Percent- age rise
		mm. Hg	mm. Hg				mm. Hg	mm. Hg	
19	Intact	114	16	14	29	Bi-lateral Nephrectomy	140	45	32
18		122	10	8	28		176	30	17
20		162	8	5					
					34		118	24	20
25		112	28	25	33		120	28	23
21		122	10	8	31		134	30	22
24		142	38	27	32		138	42	30
22		180	6	3	36		160	26	16
23		192	14	7					

RESULTS

In table 1 summarized blood pressure responses from the first 1.0 cc. injection of standard renin are presented. The maximal elevations of blood pressure of the 8 intact dogs during the first three minutes after injection of renin varied from 3 to 27 per cent, and of the 7 nephrectomized dogs, from 16 to 32 per cent. The greater responses of the nephrectomized dogs were in line with what might be expected if the current belief that the liver produces hypertensinogen more or less continuously be accepted, for the removal of the kidneys would take away the source of renin which normally reacts with the hypertensinogen, therefore, allowing the hypertensinogen to increase in the blood.

In table 2 summarized blood pressure responses from the second 1.0 cc. injection of standard renin are presented. The maximal rise in blood pressure of the 3 intact dogs not hemorrhaged varied from 2 to 8 per cent, while the maximal responses from the 2 nephrectomized dogs, which were not bled, to the second injection were 12 and 20 per cent.

The dogs subjected to hemorrhage both intact and nephrectomized, on the contrary, gave profound blood pressure responses to the second injection of renin, the

rises being much greater than those elicited by the first injection in spite of the fact that some tachyphylactic reduction in response might have been expected. The second injection of renin in the dogs subjected to hemorrhage, i.e. the injection immediately following hemorrhage, produced rises in blood pressure varying between 29 and 148 per cent in the intact dogs and 73 and 182 per cent in the nephrectomized dogs.

TABLE 2. BLOOD PRESSURE RESPONSES FOLLOWING SECOND INJECTIONS OF 1.0 CC. HOG RENIN

DOG	KIDNEY CONDITION	HEMORRHAGE	TIME FROM START OF HEMORRHAGE TO 2d. RENIN INJECTION, MIN.	BLOOD PRESSURE		
				Initial	Actual rise	Percentage rise
				mm. Hg	mm. Hg	
19	Intact	None		126	10	8
18				136	6	4
20				176	4	2
25		Hemorrhage	20	64	34	53
21			15	62	18	29
24			20	50	74	148
22			10	72	30	41
23			17	52	28	54
29						
28	Bi-lateral Nephrectomy	None		146	30	20
				180	22	12
34		Hemorrhage	30	54	62	113
33			35	44	32	73
31			40	48	66	138
32			25	44	80	182
35			30	54	70	130

DISCUSSION

From these pronounced elevations in blood pressure in both intact and in nephrectomized dogs following hemorrhage, within 16 to 32 minutes after the initiation of hemorrhage, it seems that immediately following hemorrhage during the readjustments to the loss of blood and to the resultant hypotension that the supply of blood hypertensinogen is abruptly increased. This increase in the hypertensinogen content of the circulating blood might be produced by a return to the general circulation of blood from the liver, the source of hypertensinogen, during the vasoconstrictor reactions which return blood from all of the visceral organs, or as a result of increased production of hypertensinogen by the liver.

This sudden rise in hypertensinogen noted immediately after hemorrhage in both intact and in nephrectomized dogs might be interpreted as a very rapid compensatory response to make the increased out flow of renin from the kidneys, which are stimulated by the post-hemorrhage hypotension and anoxemia, available for production of hypertensin at the time of circulatory crisis. This hypertensin could act beneficially against the existing hypotension.

SUMMARY

A rapid and marked rise in the hypertensinogen content of blood immediately after hemorrhage, that is within 16 to 32 minutes after the hemorrhage started, was demonstrated by the injection of standardized hog renin into both intact and nephrectomized dogs. The possible application of this rise in blood hypertensinogen to the circulatory crisis following hemorrhage is discussed.

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ENZYME STUDIES ON HUMAN BLOOD. IV. INTERRELATION OF HEPARIN AND FIBRINOGEN FRACTIONS¹

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INVESTIGATIONS on the possible mechanism of the anticoagulant action of heparin have centered largely on the problem of its cofactor. In 1918, Howell and Holt (1) showed that heparin requires a blood factor to prevent the coagulation of fibrinogen by thrombin. Subsequently Quick (2) suggested that an albumin fraction contains normal antithrombin, which together with heparin, exerts a powerful anticoagulant action. Others (3, 4, 5) confirmed that the heparin cofactor is a substance associated with an albumin fraction, but not crystalline albumin.

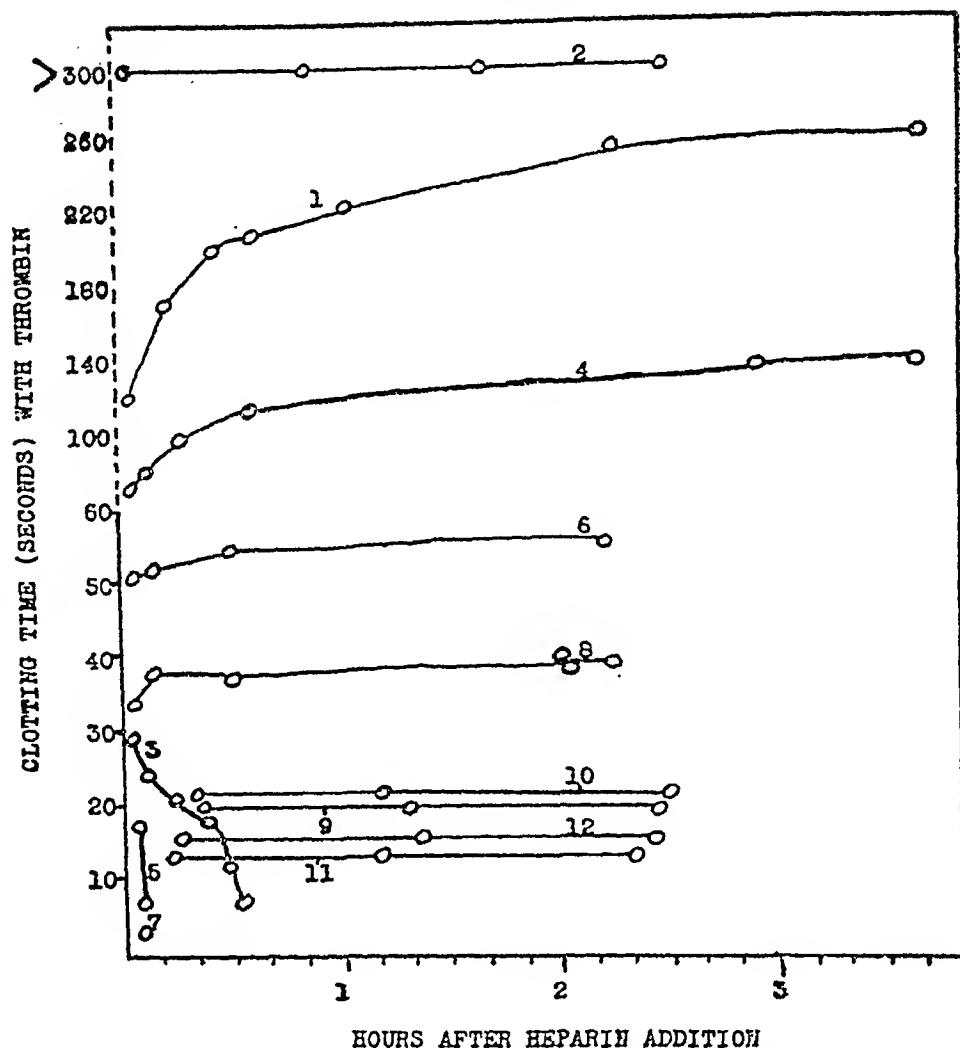
In a previous communication from this laboratory (6) it was demonstrated that human plasma albumin actually accelerates the reaction of fibrinogen fractions with thrombin. Therefore, in view of this finding and the reported synergism of antithrombin and heparin, experiments on the influence of heparin on fibrinogen fractions were undertaken.

EXPERIMENTAL

Fraction I was prepared by the low temperature-ethanol procedure, Step 6, of Cohn *et al.* (7). Subfractions which had a fibrinogen purity of 70 to 80 per cent were prepared by lowering the temperature of a concentrated Fraction I solution to 0° (8). Ethanol was added to the resulting 0° supernatant at pH 7.2 $\Gamma/2$, 0.129, to a final concentration of 8 per cent, at final temperature of -2° to -3°. The precipitate was separated in a refrigerated centrifuge and dried by lyophilization. The dried powder was dissolved in citrate-phosphate buffer (pH 7.2 $\Gamma/2$, 0.129) to yield a 1 per cent solution. The insoluble material after 20 minutes of careful stirring was removed by centrifugation at 1000 g, 20°, for 30 minutes. The supernatant fluid was opalescent and showed no fine or large particles. Approximately one half of this solution was filtered once through a Seitz filter (50 cc/6-cm. pad). Spectrophotometric data at 450 m μ , for relative values only were obtained on the filtered (F) and not filtered (N) solutions. The preparation of thrombin, buffer and albumin solutions, and the technics for the determination of clotting time, protein and clottable protein have been previously described (6, 9). The results from the determination of fibrinogen purity as done in this laboratory are at best approxima-

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¹ Fibrinogen fractions were prepared in this laboratory from dried normal plasma processed from blood obtained from volunteer donors enrolled by the American Red Cross.



HOURS AFTER HEPARIN ADDITION

Fig. 1. INFLUENCE of HEPARIN, albumin, and incubation time on Seitz-filtered (F) and not filtered (N) fibrinogen fractions.

CURVE NO.	MIXTURE ¹					OBSERVATIONS WITHOUT ADDED THROMBIN
	Fibrinogen Stock ²		Buffer	4% Al- bumin	Heparin Added	
	(N)	(F)				
	cc.	cc.	cc.	cc.		
1	3		6		3 cc. 40 mg.-%	Opalescent: no change, 6 hrs.
2		3	6		" " " "	Clear: no change, 6 hrs.
3	3			6	" " " "	Opalescent: granules, 1 hr.
4		3		6	" " " "	Clear: no change, 6 hrs.
5	3		6		3 cc. 8 mg.-%	Granules, 4 min.: pellicle, 90 min.
6		3	6		" " " "	Clear: no change, 6 hrs.
7	3			6	" " " "	Granules, 1 min.: pellicle, 40 min.
8		3		6	" " " "	Clear: no change, 6 hrs.
9	3		9		None	Opalescent: no change, 6 hrs.
10		3	9		"	Clear: no change, 6 hrs.
11	3		3	6	"	Opalescent: no change, 6 hrs.
12		3	3	6	"	Clear: no change, 6 hrs.

¹Clotting times with thrombin were done on 0.8 cc. aliquots.

²See Exp. 1, table 1, for other data.

tions. The factor 16 for the relative chromogenic values of protein to tyrosine with the phenol reagent was obtained on Fraction I. It does not follow that this factor would also be applicable to both the clottable and nonclottable components of fibrinogen fractions. However, for comparative purposes this method was employed, since insufficient samples made gravimetric estimations of clottable protein as obtained by thrombin or by heat impossible.

Proteolytic enzyme was estimated as outlined in another report (10) with one exception. The preparation of the substrate was modified in view of the report that traces of a proteolytic enzyme occur even in C.P. casein (11). A 4 per cent casein (Pure, Coleman) in 1/10 M Na_2HPO_4 was heated at 80° for five minutes. One volume of this inactivated material was added to one volume of buffer, the resulting substrate being 2 per cent casein in 1/15 M Sorensen buffer at pH 7.3. With this modification, 100 cc. of the 2 per cent substrate control after 48 hours incubation contained 2.30 mg. \pm S.D. 0.019 acid soluble 'tyrosine' in five separate runs in contrast to variable results as high as 14 mg. found in the previous work (10). For a negative enzyme control, the action of albumin (Fraction V) on casein was tested: the results were -0.48 mg. \pm S.D. 0.27 acid soluble tyrosine (five separate runs) per 100 cc. 2 per cent albumin solution.

All solutions except the citrate-phosphate buffer were made up on the day of the experiment. The legend for figure 1 is an example of a protocol. Heparin (Connaught Laboratories, 110 Toronto U/mg.) was dissolved in the buffer just before addition at zero time. Clotting time estimations were done on 0.8-cc. aliquots of the resulting mixtures by the addition of 0.2 cc. 1-10 'Hemostatic Globulin' (Lederle).

The calcium content of the dried fibrinogen fraction was determined on a 1.5 m grating spectrograph (Applied Research Laboratories). Ultraviolet absorption studies on filtered and not filtered fibrinogen fraction solutions were done in a quartz spectrophotometer (Beckman, Model D.U. with ultraviolet accessory).

RESULTS

The influence of heparin on the fibrinogen fraction-thrombin reaction in relation to incubation time and added albumin is shown in figure 1. The control curves 9, 10, 11 and 12 demonstrate that there are no significant differences in the thrombin clotting times between filtered and not filtered fibrinogen fractions, that there are no changes in the values obtained during a test period, and that the addition of albumin lowers the clotting time (6). High concentration of heparin (10 mg. %) definitely increases the clotting time. In the filtered fraction (curve 2) the increase is much greater than in the not filtered fraction (curve 1). Addition of albumin to the fraction before heparinization diminishes this increase in the clotting time in the filtered fraction (curve 4) and much more so in the not filtered fraction (curve 3). Addition of less heparin (2 mg. %) increases the clotting time to a lesser degree in the filtered fraction (curve 6), but reduces the clotting time in the not filtered fraction (curve 5). The addition of albumin depresses the increased clotting time of the filtered fraction (curve 8) and leads to immediate clot formation with thrombin in the not filtered fraction (curve 7).

Observations on the effect of heparin alone on fibrinogen fractions, without

measurements with thrombin, are recorded in the legend for figure 1. Large granules or threads appeared in less than five minutes in this and other experiments in not filtered fibrinogen fractions at the 2 mg. per cent heparin concentration level. By vigorous shaking the large granules could be broken down into finer granules, but in 10 to 20 seconds they would reform. In three out of five experiments 'clots'

TABLE 1. EFFECT OF VARYING HEPARIN CONCENTRATIONS ON FILTERED AND NOT FILTERED FIBRINOGEN FRACTION

PREP. NO.	EXP. NO.	DATA ON FIBRINOGEN FRACTIONS WITHOUT ADDED HEPARIN						RELATIVE CLOTTING TIME ONE HOUR AFTER ADDING HEPARIN ⁵ $28 \pm 0.5^\circ$ mg. heparin/100 cc. solution						
		Stock solution				Proteol. En- zyme ³	Clotting time ⁴ Sec. \pm S.D.	10	2	1	0.4	0.08	.016	.003
		N/F ¹	% Pro- tein	% Pur- ity fib- rinogen	2-Log T 450 mu ²									
R ₂ S	1	N	1.04	72	.959	9.2	20.5 \pm 0.4	10.0	0.25					
		F	.73	70	.065	-2.1	21.3 \pm 0.4	> 20	2.85					
R ₃ S	2	N	1.07	80	.745	16.2	20.0 \pm 0.9	> 20		2.79		2.10		
		F	.73	76	.048		20.5 \pm 1.8	> 20		7.20		2.39		
S ₅ S	3	N	.97	76	.538	28.3	32.3 \pm 1.1	18.0	0.15		1.04	1.64	1.24	1.00
		F	.75	74	.065	2.8	35.0 \pm 0.5	> 20	1.90		2.04	2.00	1.42	1.09
S ₅ S	4	N	.80	75	.509		17.9 \pm 0.8	7.1	0.77		1.85	1.91	1.23	1.03
		F	.60	70	.041		17.6 \pm 1.3	> 20	3.22		2.82	3.10	1.77	1.17
E ₁₅ S	5	N	.96	70	.523	16.0	24.1 \pm 0.6	1.5	0.24		2.30	1.50	0.90	0.99
		F	.74	74	.065		23.8 \pm 0.2	7.6	2.10		2.35	2.18	1.30	1.00
S ₄ ⁶	6	N	.90	60	.545	11.5	19.5 \pm 1.4	5.6	1.60		2.24	1.22	1.04	0.99
		F	.76	62	.091	1.5	19.7 \pm 0.4	7.6	2.30		2.42	1.80	1.11	1.07

¹ N, not filtered; F, filtered (Seitz).

² 19 X 105 mm. cuvette, Coleman Junior Spectrophotometer.

³ Net mg. acid soluble 'Tyrosine' released by 100 cc. 2% solution, pH 7.3, 48 hrs., 37.5°C.

⁴ 0.2 cc. 1-10, 'Hemostatic Globulin' (Lederle) plus 0.8 cc.; .15-.26% fibrinogen fraction: citrate-phosphate buffer, 1/2 0.129, pH 7.2: Mean of more than 3 tests /3 hours. (outdated thrombin used in Exp. 3).

⁵ Clotting times as in *ftnt.*⁴ In some cases, the one hour value was obtained by interpolation of 4 to 6 results obtained at different times. Relative clotting time =

$$\frac{\text{C. T. fibrinogen Fraction} + \text{heparin.}}{\text{C. T. fibrinogen Fraction alone}}$$

C. T. fibrinogen Fraction alone

⁶ Fraction I. The other preparations are subfractions.

appeared within 30 to 90 minutes. These 'clots' resembled the pellicle occasionally observed in the spinal fluid of tuberculous meningitis. In similar experiments with added albumin, granules and clot formation occurred more rapidly.

Table 1 represents a summary of six experiments with Fraction I and its sub-fraction of higher fibrinogen concentration. In each of the experiments as many as 28 reaction mixtures were tested. Since it would not be feasible to report 140 curves

of the type depicted in figure 1, the relative clotting times one hour after the addition of heparin as well as other data in the six experiments are summarized.

The anticoagulant activity of heparin at 10 mg. per cent concentration without added cofactor is demonstrated in all six experiments: In tests with filtered fibrinogen fractions the clotting times with thrombin are 7 to over 20 times greater than those of their respective controls; with not filtered fibrinogen fractions, $1\frac{1}{2}$ to 18 times greater. At a lower (critical) concentration of heparin an opposite activity is observed in all five experiments on subfractions of Fraction I. The clotting times of not filtered fibrinogen fractions with thrombin are *lower than those of their respective controls*, and this phenomenon could be observed usually in less than one hour. The anti-coagulant effect is again definitely present at still lower heparin concentrations, and disappears finally. The results from the other added albumin experiments have

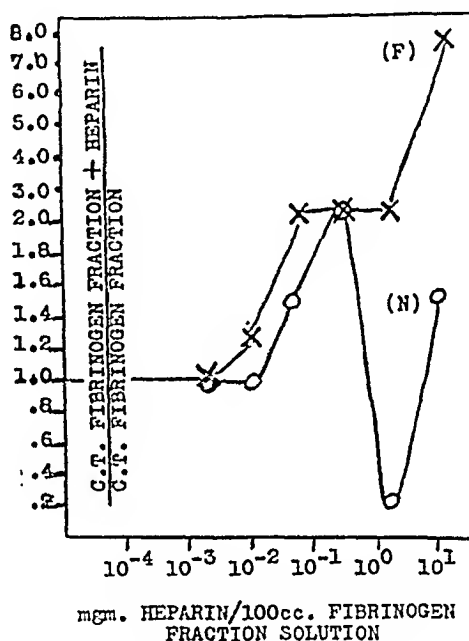


Fig. 2. INFLUENCE OF HEPARIN concentration on the clotting times with thrombin of Seitz filtered (F) and not filtered (N) fibrinogen fractions. See *Exp. 1*, table 1, for other data.

been omitted since the data in figure 1 are typical of all experiments; albumin definitely decreases the thrombin clotting times of all reaction mixtures tested, with or without added heparin, on filtered or not filtered fibrinogen fractions.

Proteolytic enzyme activity (10) was present in all preparations studied. The almost complete removal of this enzyme activity by Seitz filtration and/or adsorption was also noted. It was found by spectroscopic analysis that there was less than 6×10^{-6} M of calcium in 0.25 per cent fibrinogen fraction. Ultraviolet absorption studies showed a maximum extinction at 280 μ , and minimum extinction at 254 μ in both the filtered and not filtered solutions (0.05% protein, pH 7.2).

Figure 2 shows the relative clotting times plotted against heparin concentrations. Curve F is obtained with filtered fibrinogen fraction, Curve N, with the not filtered fraction. Similar curves characteristic for the filtered and not filtered preparations were also obtained in experiments 3 and 4. In experiment 6, Fraction I—Prep. S4,

the curves are likewise comparable to those in *experiment 5* except that the clotting time values do not reach low levels as in the other experiments.

DISCUSSION

The results of the foregoing experiments suggest the existence of a biologically active substance or substances which for the purpose of this discussion shall be designated Factor P, and which may be characterized as follows: it is present in Fraction I, but more evident in subfractions; it can be mostly removed by filtration with the Seitz filter and/or concomitant adsorption; it does not perceptibly influence the ultraviolet absorption of fibrinogen fractions; it has a complex influence upon the clotting mechanism of fibrinogen fractions depending on the heparin concentration.

The totally diverse effects induced by the addition of various concentrations of heparin to not filtered fibrinogen fractions require some comment. The following is suggested as an interpretation of this phenomenon. Where H is the active anticoagulant (heparin, heparin complex, or the active heparin moiety), and P is Factor P as described above, at a certain concentration level of added heparin an anticoagulant effect is produced: $P + nH \rightleftharpoons PH_n$. As more heparin is added, a second equilibrium is established, showing a coagulant effect: $PH_n + mH \rightleftharpoons PH_{n+m}$. Since the maximum amount of PH_{n+m} has been formed, additional heparin again results in an anticoagulant activity. (The methal ammine reaction of Ag with variable concentrations of NH_3 is cited as a chemical analogy to the above equilibria.) This concept is compatible with the results obtained in similar experiments with once Seitz-filtered fibrinogen fractions, which can be considered systems deficient in Factor P. The plateau on the heparin concentration-clotting time curve (fig. 2) is interpreted as evidence of a minute amount of Factor P not removed by Seitz filtration.

In consideration of the probable identity of Factor P, it is emphasized that the total calcium concentration is less than $6 \times 10^{-5} M$ in the 0.25 per cent fibrinogen fraction solutions reported here. This amount is about 4 per cent of the $1.5 \times 10^{-5} M$ of ionized calcium, the minimum necessary to activate prothrombin in the presence of excess thromboplastin, according to Nordbo (12) and Quick (13). This fact then would rule out thromboplastic substances requiring ionized calcium to activate prothrombin. It has been reported that trypsin can activate prothrombin in a calcium ion-free system (14). The fact that proteolytic enzyme activity (10) is removed by Seitz filtration and that prothrombin can be removed by the same process is not evidence that they may be components of Factor P but serves only as a guide in future experiments. The relation of Factor P to antihemophilic globulin (15), also present in Fraction I, is being studied.

On the basis of electrophoretic studies, Chargaff *et al.* (16) suggested that the most likely point of attack of heparin in plasma is the beta globulin. They observed that a globulin fraction with the mobility of gamma globulin was not attacked by heparin. These observations are of interest in view of the results in the present work with fibrinogen subfractions. Further purification of Fraction I removes albumin and alpha globulins before beta and gamma globulins (7, 17).

That concentrations as low as 0.01 mg. (1.1 Toronto units, 5 Howell units) heparin can influence the clotting time with thrombin of 100 cc. of filtered fibrinogen fraction solution is of significance (table 1, *Exp.* 3, 4, 5). This amount is not far removed from the 2.4 mg. crude heparin (0.4 Howell units) found in 100 gm. of ox serum by Charles and Scott (18) with an isolation procedure, which for comparative purposes may be satisfactory but for the estimation of the absolute concentration is open to question. In another communication (9), the anomaly of increasing purity of fibrinogen fractions resulting in a higher clotting time with thrombin was explained on the basis of a possible concomitant removal of coagulant and/or concentration of anticoagulant factors in the purification procedure.

In conclusion, the results obtained thus far in this laboratory are indicative of a system in which Factor P and heparin interact and are held in delicate equilibria. This theory suggests a reconsideration of Howell's (19) concept that heparin is a normal stabilizer of the fluidity of blood.

SUMMARY

The effect of heparin on fibrinogen fractions, prepared by low salt-low temperature-ethanol procedure, was studied. As low as 0.01 mg. heparin, without added cofactor, has a measurable anticoagulant effect on 100 cc. of Seitz-filtered fibrinogen fraction. On not filtered fibrinogen fraction solutions, variable but reproducible effects, depending on the concentration of added heparin, were obtained. This phenomenon suggests the presence of another factor (P) which is necessary for the production of the coagulant effects of heparin in fibrinogen fractions.

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EFFECT OF INCREASED RENAL VENOUS PRESSURE ON RENAL FUNCTION¹

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THE formation of edema in cardiac failure was assumed for many years to be secondary to an increase in venous pressure. According to this hypothesis the major factor causing water and salt retention was extrarenal; the increased hydrostatic pressure forced fluid out of the vascular compartment into the tissue spaces and thus made this fluid unavailable for excretion. The lesser, or renal factor, leading to the oliguria of cardiac failure was supposedly the result of renal congestion caused by the increased venous pressure (1). In 1913 Rowntree, Fitz and Geraghty (2) studied the effects of chronic passive congestion of the kidney on renal function in dogs. They applied a band about the left renal vein and collected urine from the two kidneys separately through ureteral catheters. They observed in some dogs little change in urine flow on the left but a 20 to 40 per cent reduction in phenol-sulphonphthalein (PSP) excretion and a diminished urine chloride concentration and chloride output. When they performed a right nephrectomy and then banded the left renal vein, there was a reduction in salt output without change in PSP excretion and no consistent change in urine flow.

In 1937 Winton (3) reviewed the physical factors governing urine flow. On the basis of his experiments in animals, as well as the work of others, he concluded that

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compression of the renal vein with subsequent renal congestion leads to a reduction in urine flow and chloride excretion. The findings were believed to result from a partial obstruction to the flow of urine through the tubules by the increased intra-renal pressure. Studies have been conducted on the effects of increased intra-abdominal pressure on renal function (4-7). Reduction in urine flow and sodium excretion (8) have been demonstrated but the results are difficult to evaluate since the pressure in the renal pelvis as well as the pressure in the renal vein were increased.

Since the introduction of renal-clearance techniques the mechanisms involved in the renal retention of sodium in cardiac failure have received considerable attention. Warren and Stead (9), Merrill (10), Mokotoff, Ross and Leiter (11) and Stead, Warren and Brannon (12), have suggested that in many patients reduced sodium excretion is related to a reduction in glomerular filtration rate, especially following muscular activity (13). Other workers believe that the decreased sodium excretion is not entirely related to filtration rate but is related to an increased rate of sodium reabsorption (14-17). In accordance with classical concepts elevated venous pressure has been suggested as a possible cause (14, 18). The present studies were undertaken to determine by the newer techniques available what effect the single variable of increased renal venous pressure has on renal function.

METHODS

Twenty-two male or female dogs weighing 10 to 32 kg. were used. They were anesthetized with sodium pentobarbital administered intravenously in doses of 30 to 35 mg/kg. Renal function was measured separately and simultaneously in the two kidneys by means of clearance techniques. During the experiment the pressure was raised only in the left renal vein. The right kidney thus served as a control.

Certain operative procedures were necessary to prepare the animal for study. The abdomen was opened by midline incision. Both ureters were cannulated with plastic tubing in order to collect urine from the two kidneys separately. The left renal venous pressure was measured with a saline manometer through a no. 9 or 10 venous catheter which had been inserted through a small incision into the right jugular vein and thence manipulated into the left renal vein. A constant infusion of isotonic saline solution flowed slowly through the catheter to prevent blockage by blood clot. The pressure in the left renal vein was raised by means of a specially constructed screw-clamp. Extension arms on the clamp made it possible to compress the renal vein and release the compression without disturbing the abdominal contents.

When the operative procedure was completed, urine was collected separately from the two kidneys during periods of equal duration ranging from 5 to 20 minutes. After two or three initial control periods the pressure in the left renal vein was raised by 50 to 420 mm. saline above the initial level which ranged around 100 mm. saline. The elevation in venous pressure was maintained for two to four periods. The clamp was then released and renal function studied for two or three additional periods at the control venous pressure. The mean arterial blood pressure was measured with a mercury manometer connected to the left common carotid artery. Left renal venous pressure and mean arterial blood pressure were recorded every 5 minutes.

The following clearance techniques were used. The creatinine clearance (C_{Cr}) was considered equivalent to the glomerular filtration rate and the clearance of para-

aminohippurate (C_{PAH}) was considered an adequate estimate of renal plasma flow. Water excretion was recorded in some experiments as the percentage of filtrate excreted as urine, i.e. the urine flow divided by the filtration rate (V/C_{Cr}). Sodium excretion has been treated similarly, the sodium clearance divided by the creatinine clearance (C_{Na}/C_{Cr}) giving the percentage of filtered sodium excreted in the urine. Therefore $1 - C_{Na}/C_{Cr}$ is the percentage of filtered sodium reabsorbed. Glucose $Tm(Tm_G)$ and diodrast $Tm(Tm_D)$ were measured in 2 dogs to evaluate tubular function.

With few exceptions each dog received during the operative procedure an amount of 1.5 or 2.0 per cent saline solution equal to 5 per cent of its body weight. This infusion was given in about 30 minutes to one hour through a cannula inserted into the right femoral vein and was followed by the administration of appropriate doses of creatinine, para-amino-hippurate (PAH), glucose or diodrast to attain blood concentrations within a suitable range, viz. 20 to 40 mg. per cent, 0.8 to 2.5 mg. per cent, 450 to 950 mg. per cent and 20 to 50 mg. per cent, respectively. These concentrations were maintained at a relatively stable level by the administration of a sustaining infusion at a constant rate of 2.7 to 3.0 cc. per minute. The amounts of the drugs for the initial dose and the infusion were calculated on the basis of the weight of each dog.⁴ Fifteen or 20 minutes after starting the sustaining infusion or after the last surgical manipulation, depending on whichever came later, the collection of urine samples was begun. Eight to 15 cc. of blood were withdrawn every 15 to 30 minutes through a retention needle inserted into the right femoral artery. Potassium oxalate was used as an anticoagulant. Blood samples were centrifuged and the plasma separated. Creatinine, PAH, glucose and diodrast were determined in plasma filtrates made with $CdSO_4$ according to the method of Fujita and Iwatake (19). Urine samples were collected directly in graduated cylinders and prepared for analysis by suitable dilutions. Creatinine was determined by a modification of Bonsnes's method (20), PAH by the method described by Goldring and Chasis (21), glucose by the Nelson-Somogyi method (22) and diodrast by Alpert's method (23), modified in this laboratory by Fithian and Baker (24). Sodium analyses were carried out on an internal standard type flame photometer.

RESULTS

The 13 dogs⁵ in which experimental procedures were satisfactory have been divided into 4 groups. In *Group I* were those dogs in which venous pressure was

⁴ In dog 26 no sodium was administered; both initial and sustaining infusions consisted of a solution of 10% dextrose in water. In dog 23 the amounts of water and sodium administered were limited. The initial infusion was an amount of 0.85% saline solution equal to 3% of the body weight and the sustaining infusion, likewise isotonic saline solution, was given at a rate of 2.6 cc/min. In dogs 22 and 27 glucose Tm and in dog 27 diodrast Tm were determined. These dogs received initially an amount of isotonic saline solution equal to 8 to 10% of body weight. The sustaining infusion of isotonic saline solution, which contained 15% glucose and 1.8% diodrast, was administered at a rate of 5 to 6 cc/min.

⁵ Clearance values were obtained on 22 dogs but the results in 7 were not considered valid because the filtration rate fell off more than 25% during the course of the experiment. The mean arterial blood pressure also gradually declined, usually to below 100 mm. Hg. Glucose Tm was determined in 2 of the 15 satisfactory experiments. The control values for C_{PAH} , C_{Cr} and filtration fraction per square meter of surface area were somewhat higher than those obtained by Selkurt (25)

raised to between 100 to 200 mm. saline, in *Group II* those in which venous pressure was raised to between 200 to 300 mm. saline and so on. With three exceptions the initial pressure was 100 ± 20 mm. saline. It has been assumed that the control pressure in the right renal vein was approximately the same as the control pressure in the left. The classification of the experiments into these 4 groups has been maintained throughout the presentation of the data in figures 1 through 6. In these figures the abscissa is divided into three sections. The first comprises the three control periods of urine collection at the initial venous pressure of 80 to 120 mm. saline. The second section contains five segments, each representing a different increment in venous pressure. Each of the five segments is subdivided into the three separate, successive periods of urine collection obtained while the venous pressure was elevated. Hence, it is possible to illustrate the effect on renal function of the duration of the increase in venous pressure as well as the effect of the height of the venous pressure. The third, or recovery section, comprises the three periods of urine collection following return of the left renal vein pressure to its control value. The results observed in any one dog will fall into the control section, one of the five segments of the increased venous pressure section, and into the recovery section. On the ordinate in all six figures are plotted the values which represent the function of the left kidney as compared to that of the right. The values representing the relative function of the left kidney are not the actual value differences between the two kidneys but are the percentages that the function in the left kidney differed from the same function in the right. For example, if the plasma flow through the left kidney was 105 cc/min. and that through the right kidney was 100 cc/min., the value plotted on the ordinate would be $\frac{105}{100} \times 100$ or 105 per cent. The horizontal dotted lines in the figures indicate the values \pm twice the standard deviation of the mean of the control values. It is to be noted that during the three control periods of urine collection the function of the left kidney did not deviate from that of the right by more than ± 10 per cent, i.e. the function of the left was 90 to 110 per cent of that of the right. For this reason it has been assumed that any deviation of much more than 10 per cent during a period of increased venous pressure may be considered a significant deviation and attributable to the increased pressure in the left renal vein.

Effect of Increased Renal Venous Pressure on Renal Plasma Flow, Glomerular Filtration Rate and Filtration Fraction. Para-aminohippurate clearance (C_{PAH}) was measured in 10 dogs (fig. 1). Of these, 6 dogs showed no significant alteration of renal plasma flow through the left kidney despite elevation of the venous pressure up to 350 mm. saline for as long as 52 minutes. In 2 dogs C_{PAH} fell initially in the left kidney but only to a minimally significant extent and rose to control values again before the venous pressure was brought back to the control level. In 2 dogs (9 and 10), when the venous pressure was raised to 550 mm. saline, there was no initial fall in C_{PAH} but, after 15 to 20 minutes at that pressure, there was a significant drop in left renal plasma flow, 22 per cent and 66 per cent in dogs 10 and 9 respectively.

Glomerular filtration rate (C_{Cr}) was likewise essentially uninfluenced by elevation of the renal venous pressure up to 350 mm. saline (fig. 2). C_{Cr} fell slightly in

on similarly anesthetized dogs. Average figures with standard deviations were C_{PAH} 173 ± 40 cc/min., C_{Cr} 54 ± 10 cc/min. and F.F. 0.35 ± 0.06 . None of the clearances for the experiments accepted as valid fell outside twice the standard deviation of the mean.

the left kidney in one dog (No. 5) in which the pressure in the left renal vein was maintained at 350 mm. saline for 52 minutes. There were significant decreases in glomerular filtration rate of 18 and 52 per cent respectively in dogs 10 and 9 in which the venous pressure was raised to 550 mm. saline.

The filtration fraction (F.F.) was not consistently altered by the elevation of the renal venous pressure until the venous pressure was raised to as high as 550 mm. saline. It was increased about 15 per cent in dog 9 and 5 per cent in dog 10. It is possible that a greater elevation of filtration fraction was masked by so-called 'vicarious' extraction of PAH by functioning nephrons from blood which had passed through the glomeruli of non-functioning nephrons.

Effect of Increased Renal Venous Pressure on Water and Sodium Excretion. In contrast to the lack of effect of moderate increases in venous pressure on renal plasma flow and filtration rate were the marked decreases in sodium and water excretion produced by only moderate elevation of renal venous pressure. This effect on urine flow (V , fig. 3) and sodium excretion (UV_{Na} , fig. 4) was observed in one of two dogs, 23 and 25, when the venous pressure was raised to as little as 160 mm. saline. Further increments in venous pressure in other dogs decreased the excretion of water and sodium still more. In general the reduction of urine flow and sodium excretion tended to vary directly with the height of the venous pressure. Dog 13 constituted the only exception, the absence of significant effect on water and sodium excretion possibly being related to lack of parallel function between the two kidneys.

The depression of water and sodium output was either maintained throughout the periods of increased venous pressure or water and sodium loss became more marked with time, i.e. urine flow and sodium excretion from the left kidney continued to decrease throughout the three successive periods of increased venous pressure. Dog 24 was the only exception in this respect and for no apparent reason.

Following release of the clamp and return of the venous pressure to control levels the functions of the left kidney invariably returned toward control values although usually incompletely in the 10 to 20 minutes allotted. In no experiment was there a further decline in water or sodium excretion.

The changes in the percentages of filtered water and sodium excreted (V/C_{Cr} , fig. 5, and C_{Na}/C_{Cr} , fig. 6, respectively) produced by moderate increases in renal venous pressure are further evidence that the reduction in water and sodium excretion occurred independently of minor fluctuations in the filtration rate. The marked decreases in the percentages of filtered water and sodium excreted which occurred when the venous pressure was raised to above 200 mm. saline also indicate that increased percentages of filtered water and sodium were reabsorbed.

The complete data of a typical experiment are given in table 1. Elevation of the pressure in the left renal vein to 340 mm. saline caused no significant reduction in either renal plasma flow or glomerular filtration rate in the left kidney but the urine flow and the amount of sodium excreted fell markedly during the 27 minutes that the renal venous pressure was kept elevated. The figure for percentage of filtered sodium reabsorbed is considered accurate to within ± 1 per cent. Hence, during the time when venous pressure was elevated the difference of 3 per cent between the values for the two kidneys in the third period indicates that there was a significant increase in the rate of sodium reabsorption. Since, in this experiment, there was no

correlation between the percentage of sodium load reabsorbed and the magnitude of the load, it is concluded that the relative increase of 3 per cent in the left kidney was a result of the heightened pressure in the left renal vein.

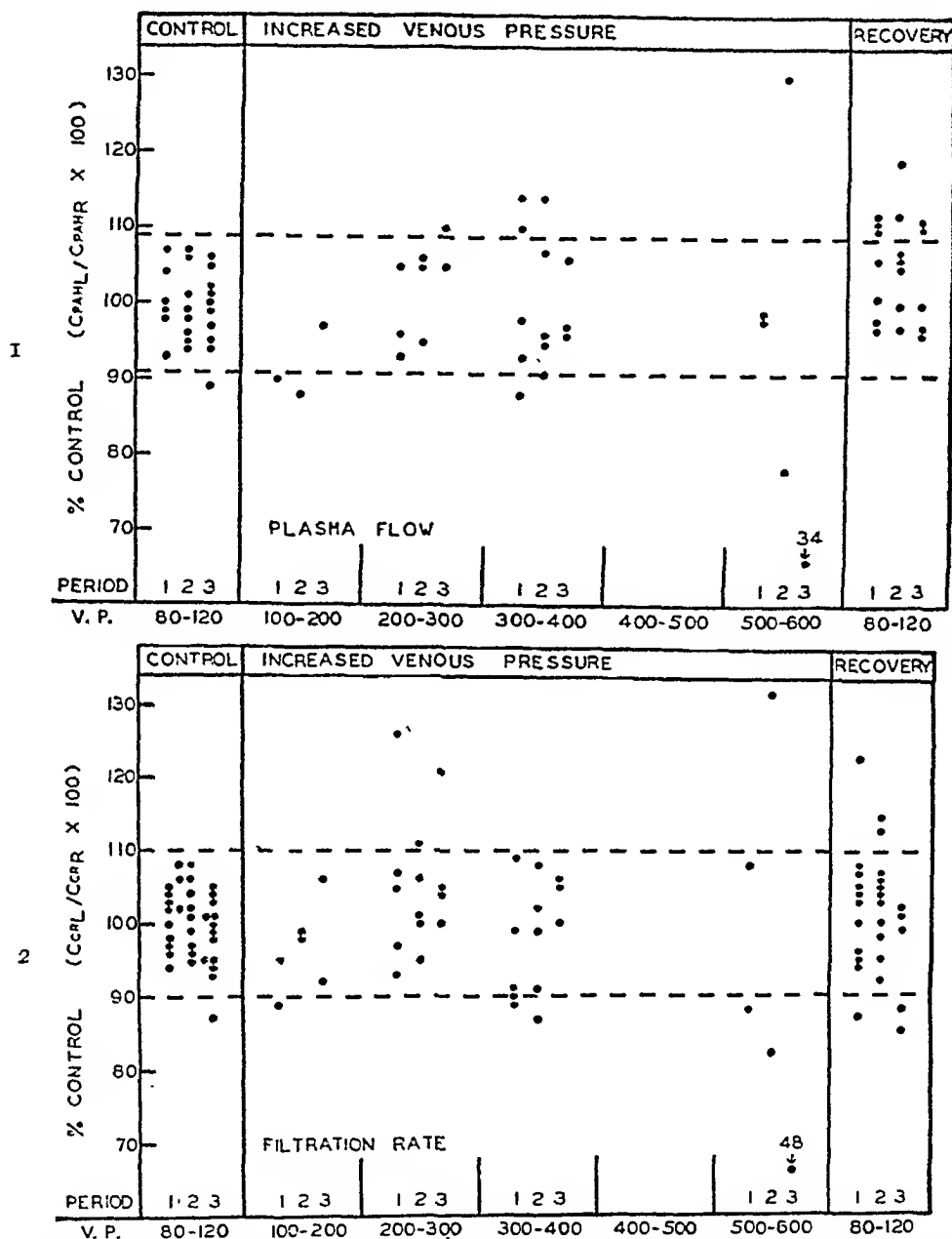
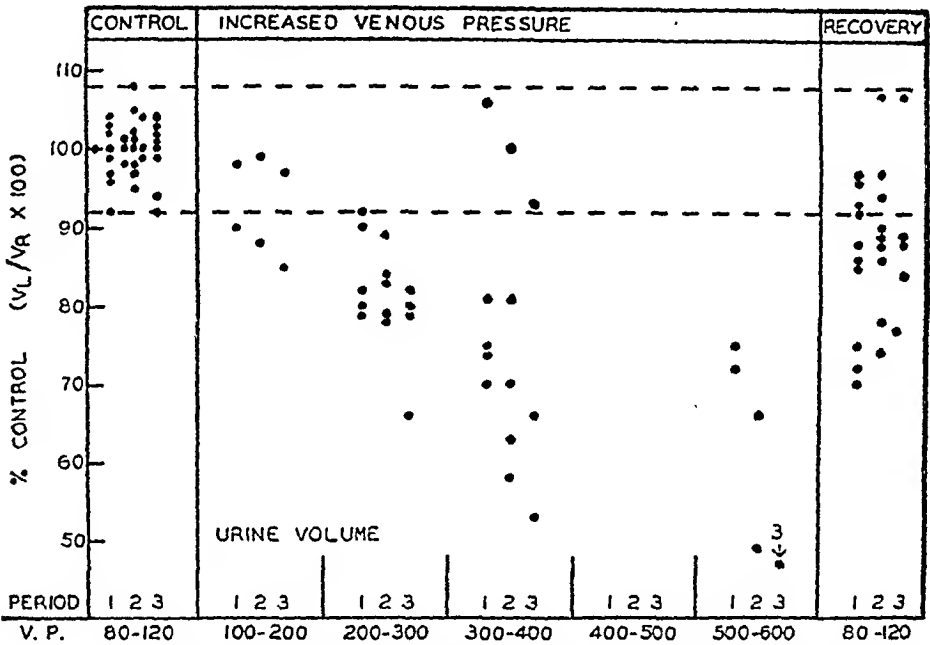


Fig. 1. EFFECT OF INCREASED VENOUS PRESSURE ON RENAL PLASMA FLOW. No significant depression of plasma flow occurred until the venous pressure was elevated to between 500-600 mm. saline.

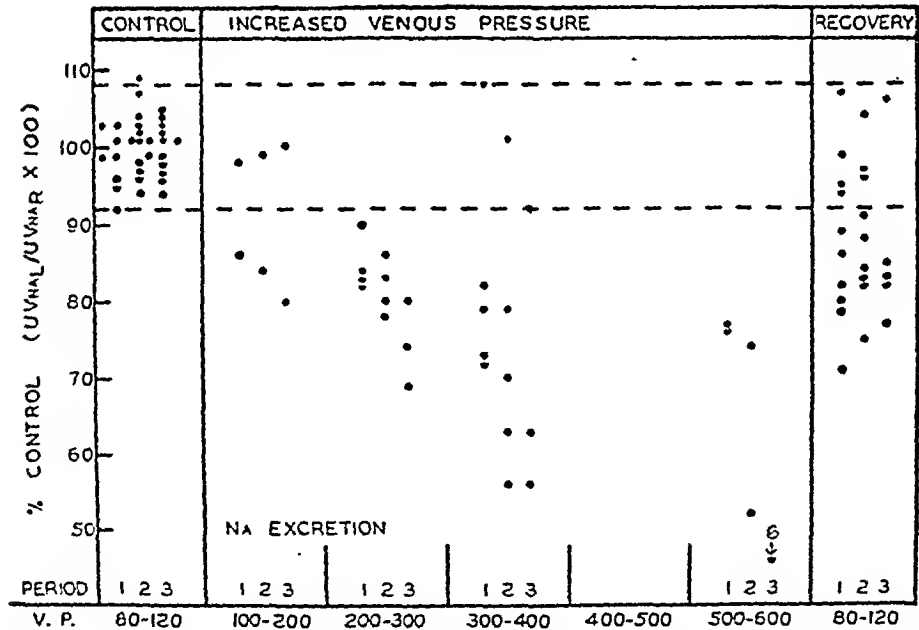
Fig. 2. EFFECT OF INCREASED VENOUS PRESSURE ON GLOMERULAR FILTRATION RATE. No significant depression occurred with venous pressure elevated up to 350 mm. saline. Reduction in filtration rate did occur in 2 dogs at 550 mm. saline pressure.

The sodium load in 11 of the 13 experiments was raised above normal by the infusion of 1.5 or 2.0 per cent saline solution. In *dog 24* the infusion was isotonic saline solution and in one other (*dog 26*) it was 10 per cent dextrose in water. In

these 2 dogs in which the sodium load was normal and below normal respectively (serum sodium was 127 mEq/l. in *dog 26*) the percentage changes in decreased water



3



4

Fig. 3. EFFECT OF INCREASED VENOUS PRESSURE ON URINE FLOW. Depression of urine flow occurred in one of 2 dogs at 160 mm. saline pressure. Greater increments of venous pressure in other dogs further depressed urine flow.

Fig. 4. EFFECT OF INCREASED VENOUS PRESSURE ON TOTAL SODIUM EXCRETED. The results are comparable to those obtained on urine flow.

and sodium excreted were comparable to those observed in the other dogs. In *dog 26* the actual decrease in sodium excretion was, of course, much smaller.

Glucose Tm was measured in 2 dogs and diodrast Tm simultaneously in one of these (table 2). In both dogs there was a decrease in urine flow during elevation of the venous pressure without any significant change in glucose Tm or diodrast Tm.

DISCUSSION

By means of the techniques employed, it was demonstrated that elevation of the renal venous pressure from a mean normal of 100 mm. saline up to 340 mm. saline

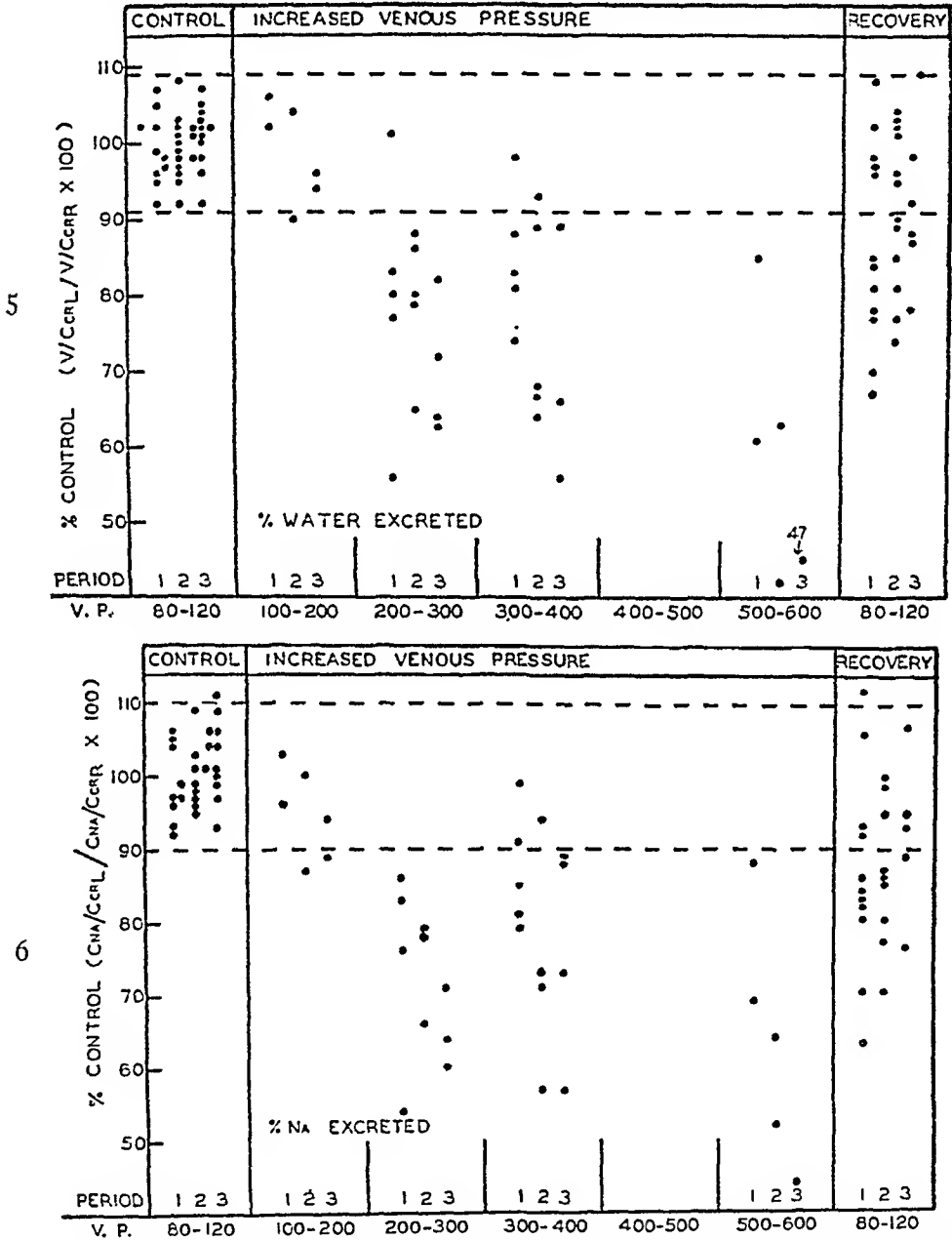


Fig. 5. EFFECT OF INCREASED VENOUS PRESSURE ON PERCENTAGE OF FILTRATE EXCRETED AS URINE, i.e., the percentage of filtered water excreted. At venous pressures above 200 mm. saline, there was depression of the percentage of filtered water excreted. This indicates that an increased percentage of filtered water was reabsorbed.

Fig. 6. EFFECT OF INCREASED VENOUS PRESSURE ON PERCENTAGE OF FILTERED SODIUM EXCRETED. The results are comparable to those obtained on the percentage of filtered water excreted. This indicates that an increased percentage of filtered sodium was reabsorbed.

had no significant effect on renal plasma flow or glomerular filtration rate. Mean arterial blood pressure also remained essentially the same throughout the course of each study. Hence, elevation of the pressure in the left renal vein decreased the

pressure gradient from renal artery to vein. The constant plasma flow in the presence of a decreased pressure gradient implies a fall in the resistance within the renal circuit. Since there was no change in filtration rate or filtration fraction, the fall in resistance probably occurred distal to the glomerulus in the efferent arteriole and/or the peritubular capillary bed.

TABLE I. RESULTS OBTAINED IN DOG 12, 20.5 KG. FEMALE

PERIOD	LEFT RENAL V. P.	V		CREATININE						PAH						F.F.	
				U		P	UV/P		U		P	UV/P					
		R	L	R	L		R	L	R	L		R	L	R	L		
	mm. sal.	cc/min.		mg. %		mg. %	cc/min.		mg. %		mg. %	cc/min.					
1	105	1.70	1.80	856	850	27.3	53	56	121	118	1.57	131	134	.41	.42		
2	"	2.26	2.78	592	533	27.7	48	54	90	80	1.52	133	146	.36	.37		
3	"	2.96	3.35	475	416	28.4	50	49	76	66	1.46	155	151	.32	.33		
4	340	3.80	3.19	378	422	28.6	50	47	59	64	1.43	156	144	.32	.33		
5	"	3.99	2.86	353	472	28.7	49	47	53	69	1.42	147	140	.33	.34		
6	"	3.38	2.38	350	550	28.8	41	46	54	78	1.41	130	132	.32	.35		
7	130	3.56	3.00	384	453	28.9	47	47	54	66	1.42	136	139	.35	.34		
8	"	3.12	2.88	432	466	29.0	47	46	59	65	1.42	131	131	.36	.35		
9	"	2.27	2.30	538	571	29.6	41	44	79	81	1.48	122	126	.34	.35		

PERIOD	ELAPSED TIME	UNa		PNa	UV/P		CNa/Ccr ¹		PNa × Ccr ²		UVNa ³		1 - CNa/Ccr ⁴		MEAN ART. PRESS.
		R	L		R	L	R	L	R	L	R	L	R	L	
	min.	mEq/l.		mEq/l.	cc/min.				μEq/min.		μEq/min.		%		mm. Hg
1	0-10	252	252	154	2.79	2.95	.052	.053	8200	8600	430	450	95	95	145
2	10-18	234	234	154	3.44	4.23	.071	.079	7400	8200	530	650	93	92	"
3	18-29	201	201	154	3.87	4.38	.078	.089	7700	7600	600	670	92	91	"
4	29-39	177	185	154	4.36	3.82	.087	.081	7700	7300	670	590	91	92	145
5	39-47	167	179	155	4.30	3.31	.088	.070	7600	7300	670	510	91	93	"
6	47-56	166	182	155	3.62	2.79	.088	.061	6400	7100	560	430	91	94	"
7	56-66	166	176	155	3.81	3.40	.080	.072	7300	7300	590	530	92	93	140
8	66-74	165	168	155	3.31	3.11	.071	.067	7200	7200	520	480	93	93	"
9	74-80	165	169	156	2.41	2.50	.058	.056	6400	6900	380	390	94	94	"

¹ Percentage of filtered sodium excreted. ² Filtered sodium (sodium load). Uncorrected for Donnan equilibrium. ³ Excreted sodium. ⁴ Percentage of filtered sodium reabsorbed. V represents urine flow; U represents urine concentration; P represents plasma concentration 2 minutes prior to the midpoint of the urine collection period; UV/P represents clearance. F.F. is filtration fraction.

When venous pressure was raised to 550 mm. saline the renal plasma flow and glomerular filtration rate were reduced. In these experiments the decreased resistance in the post-glomerular circuit was presumably insufficient to compensate for the fall in pressure gradient and the renal plasma flow decreased. The increased filtration fraction observed in these experiments indicated that a significant degree

of back pressure extended proximal to the efferent arteriole and thereby increased effective filtration pressure. Regardless of the exact quantitative hemodynamic relationships involved, it seems most probable that significant peritubular capillary congestion did occur.

The effect observed on water and sodium excretion is in contrast to that on plasma flow and filtration rate. In one dog elevation of the renal venous pressure to only 160 mm. saline resulted in an appreciable decrease in urine flow and sodium excretion. With more marked increases in renal venous pressure the effect was more pronounced. The decreased excretion of water and sodium without concomitant reduction in filtration rate indicates an increased rate of water and sodium reabsorp-

TABLE 2. EFFECT OF INCREASED VENOUS PRESSURE (V.P.) ON URINE FLOW (V), FILTRATION RATE (C_{Cr}), GLUCOSE Tm (Tm_G), AND DIODRAST Tm (Tm_D)

DOG	PERIOD	LEFT RENAL V.P.	V		C_{Cr}		Tm_G		Tm_D	
			R	L	R	L	R	L	R	L
		mm. sal.	cc/min.		cc/min.		mg/min.		mg/min.	
27	1	75	6.51	6.81	49	48	162	152	7.0	7.3
	2	75	5.98	6.30	47	49	145	152	7.2	6.6
	3	75	5.43	5.63	45	47	134	142	7.4	7.5
	4	165	4.48	4.63	48	46	148	140	7.1	7.3
	5	165	4.33	4.38	48	47	145	136	7.4	7.8
	6	205	4.30	4.10	46	47	137	144	7.5	7.8
	7	70	3.58	3.72	45	47	142	143	8.1	7.7
	8	70	3.73	3.83	47	44	147	132	7.5	7.7
22	1	105	4.35	4.56	56	65	139	179		
	2	105	4.44	4.68	56	63	152	165		
	3	105	4.09	4.38	56	63	145	136		
	4	215	3.60	3.55	55	61	142	175		
	5	215	3.20	3.16	53	55	124	133		
	6	105	2.75	2.91	50	53	122	129		
	7	105	2.71	2.88	52	52	136	128		

tion. The minor fluctuations encountered in measured filtration rate may be the result of certain technical shortcomings and/or possibly variability in the number of functioning nephrons. To minimize the influence of these minor fluctuations on the observed changes in sodium excretion the sodium to creatinine clearance ratio has been calculated for each urine-collection period. Water excretion has been handled similarly. The values for these ratios (V/C_{Cr} and C_{Na}/C_{Cr} , the percentages of filtered water and sodium excreted respectively) were also decreased by increased venous pressure, thus indicating again an increase in the percentages of filtered water and sodium reabsorbed. It might be worthwhile to point out that although small percentile changes in filtration rate may cause greater percentile

changes in UV_{Na} and hence influence C_{Na}/C_{Cr} , this explanation cannot be invoked to invalidate the interpretation of the results. It is apparent from figure 2 that during the periods of increased venous pressure the filtration rate in the left kidney was greater than that in the right as often as it was smaller. Nevertheless, with one exception, there always was observed a decrease in urine volume and excreted sodium. It seems highly unlikely that the consistent decreases in urine volume and excreted sodium in the left kidney can be ascribed to a decrease in the glomerular filtration rate.

In general, the diminution in sodium and water excreted was progressive with time and was related to the height to which the pressure in the renal vein was raised. In only one dog did a return toward the control values occur prior to returning the venous pressure to the control level. In no experiment was renal venous pressure kept up for more than 52 minutes. Therefore, it is impossible to say whether or not chronic elevation of the venous pressure would lead to chronic retention of water and sodium and other possible secondary effects such as decreased renal plasma flow and glomerular filtration rate. But, regardless of what happens to renal plasma flow and glomerular filtration rate and regardless of the mechanism involved in the decreased excretion of sodium, the important fact remains that under the stated conditions the increased venous pressure did cause a significant retention of water and sodium.

The mechanism involved in the increased reabsorption of water and sodium as a result of increased renal venous pressure is not apparent. The effect was immediate and local, i.e. limited to the kidney in which the venous pressure was raised. This seems to eliminate as a cause any humoral or central reflex mechanism. It seems probable that the effect was mechanical rather than due to any specific alteration of the intrinsic physiology of the tubular cells. The lack of change in glucose Tm or diodrast Tm tends to support this conclusion. There is no definitive evidence to indicate whether the increased reabsorption of water was independent of or dependent on the increased reabsorption of sodium or vice versa. It is possible that the peritubular capillary congestion was responsible for the increased water and sodium reabsorption either by partially obstructing the flow of urine through the tubules or by increasing the time and capillary surface area available for transfer of water and sodium.

It is impossible to say whether or not the results obtained in acute experiments on anesthetized dogs have any bearing on the pathogenesis of edema in clinical cardiac failure. Certainly the increase in renal venous pressure necessary in our studies to produce a decrease in sodium and water excretion is well within the range of venous pressures found in frank cardiac failure or even before frank cardiac failure, during exertion. Hence, the rise of venous pressure occurring in cardiac failure may very well be a factor contributing to the formation of edema in cardiac failure. The possible rôle played by the increase in venous pressure in the formation of cardiac edema does not detract from the rôle played by the reduction in renal blood flow and filtration rate in the excretion of water and sodium. However, the factor of decreased renal blood flow and glomerular filtration rate (10, 11) has been claimed to be responsible for the decreased excretion of sodium and water in cardiac failure, although the retention of sodium and water can be explained in our experiments on the basis of increased renal venous pressure alone. In terms of the Starling law of the heart,

the reduction in cardiac output occurs later in the development of cardiac failure than the increase in right ventricular pressure. In other words, the cardiac output is maintained at a normal level, everything else being equal, because the heart contains more residual blood at the end of diastole. One may wonder if the increase in the intraventricular pressure does not lead ultimately to an increase of the renal venous pressure before a decrease in cardiac output occurs.

In short, it is probable that reduction in renal blood flow and filtration rate as well as the increase in renal venous pressure plays a rôle in the formation of the edema of cardiac failure. However, the causal and temporal relationship of these two factors in the formation of cardiac edema and their relative importance remain uncertain.

SUMMARY

The effect of increased renal venous pressure on renal function was studied in anesthetized dogs by means of clearance techniques. Renal function was measured separately and simultaneously in the two kidneys but the pressure was raised in the left renal vein only, by means of a specially designed clamp. Venous pressure was measured with a saline manometer through a venous catheter which had been passed into the left renal vein. Control values for the functions of the left kidney were obtained before and after elevation of venous pressure and were comparable to controls obtained from the right kidney. Under the stated conditions moderate elevation of the left renal venous pressure up to 350 mm. saline caused in that kidney a significant decrease in water and sodium excretion without any change in the renal plasma flow, glomerular filtration rate, glucose Tm or diodrast Tm. The reduction in water and sodium excretion was due to an increase in the reabsorption rate of these substances by the renal tubule cells. This effect also occurred when the sodium load was low, for example in one experiment in which the serum sodium was 127 MEq/l. Greater elevation of venous pressure to 550 mm. saline decreased renal blood flow and filtration rate, but the results were not sufficient to state whether filtration fraction was significantly altered.

The mechanism for the increased reabsorption of sodium and water ascribed to increased venous pressure was not obvious. It was local, i.e. confined to the kidney in which the venous pressure was raised and consequently was not related to release of pituitary, adrenal and hepatic hormones. It probably was mechanical rather than the result of any alteration in specific metabolic processes of the tubular cells since there were no associated changes in glucose Tm or diodrast Tm. Some of the implications of these results with respect to the pathogenesis of edema in cardiac failure have been discussed.

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REABSORPTION OF CREATINE AND GUANIDOACETIC ACID BY THE RENAL TUBULES¹

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IT HAS been well established that creatine is formed in experimental animals (1, 2) and in human beings (3) by the methylation of guanidoacetic acid (glycocyamine). *In vitro* tissue experiments have indicated that the kidney is the predominant site of synthesis of guanidoacetic acid from arginine and glycine (4). Since its detection in normal human urine by Weber in 1935 (5) guanidoacetic acid has been consistently found in the urine of adults and children in quantities ranging from 30 to 150 mg. per day. This is in contrast to creatine, which is normally not detectable in the urine of normal adult males or in the majority of females (6). Since the liver is the most likely site of the methylation of guanidoacetic acid, the tolerance to injected and ingested guanidoacetic acid has been studied in liver disease (7-9). On the basis of preliminary observations it appeared that the ratio of endogenous urinary guanidoacetic acid to total creatinine was elevated in cirrhosis (8). During recent studies on creatine metabolism (3) it was found that the administration of creatine orally or intravenously produced a striking increase in guanidoacetic acid excretion. This suggested either an inhibition of guanidoacetic acid methylation under such conditions or a competition by guanidoacetic acid and creatine for renal tubular reabsorption. Recent studies have made the former possibility less likely (25, 10). Two conditions would have to be satisfied in order to substantiate the latter hypothesis: 1) a fall in the serum concentration of endogenous guanidoacetic acid during the administration of creatine, and 2) the demonstration by clearance techniques that the renal tubular reabsorption of guanidoacetic acid was blocked. Both of these criteria were fulfilled in the following studies.

EXPERIMENTAL

Analytical Methods. All analyses were in duplicate. Guanidoacetic acid (GAA) was determined by the method of Hoberman (11). Arginase was prepared and activated with cobalt according to the method of Hunter (12). Portions were stored at -40°C and thawed shortly before use, since a lyophilized preparation was found to lose potency rapidly. Four standards were run with each set of determinations. Since the Sakaguchi color from serum dialysates remains constant while that from standard solutions fades on warming to room temperature, readings were made at a constant interval after the addition of hypobromite. The reliability of the method was increased by sealing the margins of the dialysing membrane with a plastic cement and by extracting each 5 cc. of dialysate with 0.5 cc. of water-saturated chloroform to remove any traces of protein. Creatine produces a faint Sakaguchi reaction. Twenty mg. of creatine gave color equivalent to 1 mg. of GAA in all dilutions, and corrections were made on this basis. All urines were extracted with chloroform and in certain instances, noted in the table, were incubated with arginase. The method was found to be accurate to 5 per cent.

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Creatinine and creatine were determined by the method of Peters (13). To minimize the error introduced by the progressive darkening of the Jaffe color from serum filtrates, readings were made at a fixed 20-minute interval after addition of alkaline picrate. Since color from both pre-formed and total creatinine increases proportionally, the error in creatine determination was minimal from this source. Under the conditions of the present method and of the original Folin method, GAA is partially converted to glycohydrazide, which also give the Jaffe color. When GAA is added to standard solutions or to urine in the present method, 6 to 7 mg. of GAA are found to be equivalent in color production to one mg. of creatine. The exact ratio varies with the duration of autoclaving. In serum filtrates, however, the color production from GAA varies with the final acidity of the particular filtrate. It was found that this variation could be avoided by adding 0.5 cc. of 1N HCl to 32 cc. of the serum filtrates. In order to control each set of determinations, 7.5, 15, and 30 gamma of GAA were added before autoclaving to the filtrates from a urine and a serum sample obtained at the start of the experiment. In this manner a correction curve was established. Thymol was added to all urines as preservative. Analyses were done with a minimum of delay to avoid spontaneous conversion of creatine to creatinine and any bacterial destruction of creatinine. The creatinine determination is accurate to 5 per cent or better, while the error in determining creatine is somewhat larger, since it is estimated by difference.

Mannitol was determined by the method of Elkinton (14), the standard error of which is ± 1.2 mg. per cent for serum and ± 88 mg. per cent for urine. Fresh yeast was used in each set of determinations.

Plan of Experiments. The authors served as subjects. Experiments were run 4 to 5 hours following a creatine-free meal. 250 to 350 cc. of water per hour were ingested for 5 hours prior to and during the period of observation to obtain a urine flow of 6 to 10 cc. per minute. The subjects were not catheterized, but urine collections were delayed until a volume of over 350 cc. was obtained to minimize the effect of residual urine. The subject was kept reclining with feet horizontal, and one hour was allowed for glomerular filtration to reach a constant rate. 100 cc. of 25 per cent mannitol solution was then injected, and 35 to 40 minutes later, after equilibrium was established, the glomerular filtration rate was measured. Another intravenous injection of mannitol was given before each subsequent period to obtain comparable serum levels. During the majority of these periods, as indicated in table 1, constant infusions of GAA and creatine in physiological saline were given. The rate of infusion was precisely controlled at 480 cc. per hour by means of a tunnel clamp. These infusions were preceded by suitable booster doses given at the time of the mannitol injections. Venous blood samples were drawn with a Cournand needle at the beginning and end of each urine collection period. Before venipuncture the forearm and hand were immersed in hot water to avoid the necessity for arterial punctures.

Calculations. Glomerular filtration rates of both mannitol and endogenous creatinine were calculated in the conventional manner from the mathematical mean of the serum concentrations bracketing each period and the urinary excretion. Values were not corrected for surface area. The amount of creatine or of GAA filtered has been calculated as the product of the mean concentration in mg. per cc. of serum multiplied by the mannitol clearance in cc. per hour. The amount reabsorbed by the tubules is the differences between this value and the excretion per hour.

RESULTS

Analytical data are given in table 1.

Experiments 1 and 2 show the effect on normal GAA excretion of increasing the

amount of creatine filtered from 20 mg. per hour to 123 and 54 mg. respectively. Even at rates of filtration only slightly higher than that necessary to produce creatinuria the excretion of GAA increased and its tubular reabsorption decreased. At the same time the serum level of GAA fell 0.02 mg. per cent. This fall is consistent

TABLE 1. EFFECT OF INFUSIONS OF CREATINE AND GUANIDOACETIC ACID ON GUANIDOACETIC ACID TUBULAR REABSORPTION

EXPERIMENT	SUBJECT	INJECTION		GLOMERULAR FILTRATION RATE		GUANIDOACETIC ACID ¹				CREATINE ¹			
		Cr. ²	GAA ²	Mannitol	Creatinine	Serum conc.	Filtered	Excreted	Reabsorbed	Serum conc.	Filtered	Excreted	Reabsorbed
		gm/hr.	gm/hr.	cc/min.		mg.	mg/hr.			mg.	mg/hr.		
1	EAHS wt., 63.5 kg. ht., 72.5 in.	0	0	107	103	0.23	14.8	3.5	11.3	0.32	20.5	0	20.5
		0.25	0	100	106	0.21	12.6	6.9 ⁴	5.7	2.05	123.0	29.6	93.4
2	DWS wt., 66.0 kg. ht., 72.0 in.	0	0	91	83	0.20	11.0	2.4	8.6	0.38	20.7	0	20.7
		0.25	0	78	95	0.18	8.4	3.6	4.9	1.15	54.0	5.8	48.2
3	EAHS	0	0	119	121	0.24	17.2	4.7	12.5	0.45	32.0	0	32.0
		0	0.25	100	119	0.96	57.6	27.8	29.8	0.41	24.6	0	24.6
		0.5	0.25	96	131	0.53	30.5	27.1	3.4	0.90	52.0	5.4	46.6
4	EAHS	0.075	0	117	132	0.22	15.4	3.9	11.6	0.60	42.0	0	42.0
		0.075	0.15	91	112	0.83	45.2	21.9	23.3	0.57	31.0	0	31.0
5	DWS	0.25	0	101	93	0.16	9.7	3.3	6.4	1.44	88.6	11.7	76.9
		0.25	0.275	99	92	0.98	58.2	61.0	-2.8 ³	1.42	84.4	15.6	68.8
6	DWS	0.25	0	103	85	0.23	14.2	3.1	11.1	1.22	75.4	10.3	65.1
		0.25	0.75	105	95	2.61	165.0	158.0 ⁴	7.0	1.16	73.1	14.0	59.1
7	EAHS	0	0.5	121	101	1.95	141.0	91.0	50.0				
		0	1.5	110	107	6.71	442.0	239.0	203.0				

¹ Guanidoacetic acid and creatine filtration rates were calculated on the basis of the mannitol clearances. The results would not vary significantly were creatinine clearances used.

² Cr = creatine; GAA = guanidoacetic acid. Infusions (preceded by boosters) were given at a constant rate in the amounts indicated, yielding constant serum levels.

³ This negative value is considered within the error of the methods, and is not interpreted as indicating tubular secretion of GAA.

⁴ No arginine could be detected on incubation with arginase.

in magnitude and direction with the reduction predicted from the increased excretion, if it is assumed that GAA is distributed uniformly throughout the body water.

In experiment 3 GAA was first infused in an amount sufficient to double its tubular reabsorption, and subsequently creatine was administered. When the creatine load was thus increased, the reabsorption of GAA fell to half its initial value. *Experi-*

ment 4 serves as a control observation illustrating that when no creatinuria existed an increase in GAA filtered still increased its reabsorption.

In *experiments 5 and 6* GAA was injected in an effort to inhibit the reabsorption of creatine by the tubules. There was a moderate creatinuria of 14 to 15.6 mg. per hour in the initial periods, following which the quantity of GAA filtered was raised four-fold and ten-fold (from 9.7 mg/hour to 58.2, and from 14.2 to 165 mg/hour respectively). A moderate increase in creatinuria was found, together with a reduction in tubular reabsorption, but since tubular reabsorption of GAA was also reduced, the results are regarded as inconclusive.

In *experiment 7* the reabsorption of GAA at a relatively high serum level (6.7 mg. %) increased roughly in proportion to the amount filtered.

TABLE 2. URINARY EXCRETION OF GUANIDOACETIC ACID AND CREATINE IN NORMAL SUBJECTS AND PATIENTS WITH VARIOUS DISEASES

SUBJECT	SEX	DIAGNOSIS	GUANIDOACETIC ACID ¹	CREATINE ¹
ES	M	Normal male	68	0
DS	M	" "	41	0
HH	M	" "	66	0
AS	M	" "	52	0
BS	M	" "	62	0
JT	M	Hepatitis	72	0
AR	M	Advanced cirrhosis	61	0
WB	M	Myotonia atrophica	32	0
DW	M	Addison's disease	55	0
MN	F	Ovarian agenesis	100	40
FS	F	Normal female	69	46
ED	M	Thyrotoxicosis	83	53
TC	M	Myotonia atrophica	31	55
PP	M	Advanced cirrhosis	192	68
CB	F	Secondary syphilis	141	129
JM	M	Myotonia atrophica	93	140
AP	F	Thyrotoxicosis	255	300
MC	F	"	145	358

¹ Expressed as mg/gm. of creatinine.

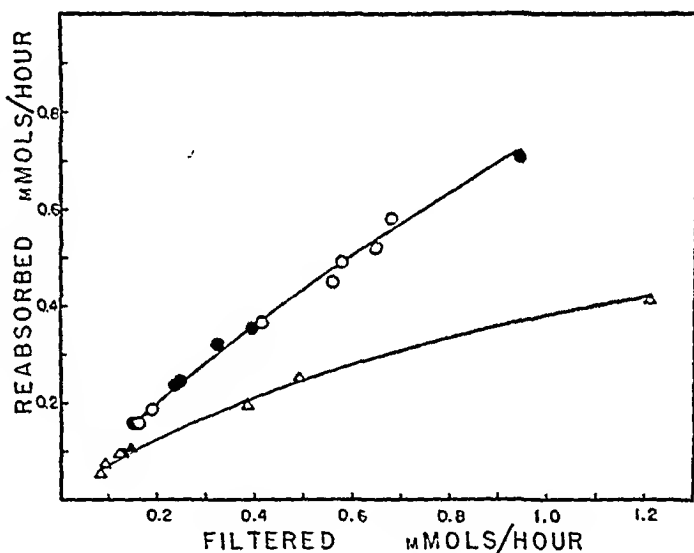
From the results above it would be expected that creatinuria from any cause should be accompanied by a high level of GAA excretion. In table 2 are summarized the results of analysis of urine from normal subjects and from hospital patients with various diseases. Urine from subjects with proteinuria was extracted with chloroform before analysis for GAA. In order to reduce them to comparable terms, the values for GAA and creatine are expressed as mg/gm. of creatinine excreted. The data are arranged in order of increasing creatinuria, and the expected correlation is evident from the figures. Three of the subjects, (*FS*, *ED*, and *TC*) do show low GAA excretion in the face of small amounts of creatine, which, however, may merely represent brief post-prandial creatinurias during the course of the urine collections. One subject with advanced cirrhosis, whose condition progressively deteriorated, shows an

excretion of GAA out of proportion to the slight creatinuria, which may indicate an impairment of methylation of GAA.

DISCUSSION

The relationship between the quantities of GAA and of creatine filtered to those reabsorbed is shown in figure 1, in which data from table 1 have been plotted. It is apparent from the figure that a smaller percentage of filtered GAA than of creatine is reabsorbed at all levels of filtration. This relative difference is more marked at higher levels of filtration. It has been possible by increasing creatine filtration within physiological limits to demonstrate a blocking effect upon the tubular reabsorption of GAA. It was not possible, however, to demonstrate the reverse effect, namely inhibition of creatine reabsorption by increase in GAA filtration. This is not surprising for two reasons. First, it is clear from a consideration of figure 1 that the reabsorptive mechanism shows less affinity for GAA than for creatine. Secondly, GAA

Fig. 1. RELATION BETWEEN QUANTITY FILTERED AND TUBULAR REABSORPTION of creatine and guanidoacetic acid. Circles represent creatine and triangles guanidoacetic acid. Hollow symbols are from subject *DWS* and solid from *EAHS*. The experiments were performed over a period of 3 months.



is always present in the urine while creatine is not found in detectable amounts, at least in adult males and in the majority of females, despite the fact that they are filtered in comparable amounts.

It is apparent from the figure that in the case of both substances, at all levels of filtration studied, less is reabsorbed than is filtered. Hence there is no evidence that either substance is excreted by tubular secretion. As the filtration of each substance increases, the quantity reabsorbed also increases, although at a diminished rate. In the case of creatine this is consistent with the finding of Zierler (15) that in normal subjects it has not been possible definitely to demonstrate a value for maximal tubular reabsorption of creatine even when serum levels are raised to 35 mg. per cent. The maximal tubular reabsorption of GAA, if such exists, has not been explored because of the magnification of error involved in estimating tubular reabsorption by difference at high levels of filtration. The constancy of the reabsorptive mechanism for the two substances is shown by the fact that values from both subjects fall on the same lines.

In calculating tubular reabsorption glomerular filtration rates were determined by mannitol. Evidence has recently been obtained from simultaneous determinations that mannitol clearances are from 0.87 (16) to 0.9 (17) smaller than inulin clearances. Were this substantiated, the absolute values for tubular reabsorption given in table 1 would be proportionally low. Since mannitol clearances are presumably proportional to actual changes in glomerular filtration rate, however, the relative changes are unaffected.

Evidence has been advanced suggesting that substances other than GAA compete with creatine for a common tubular transport system. Pitts has presented data which suggest a blocking of the tubular reabsorption of creatine by glycine (18), by alanine and to a lesser extent by glutamic acid (19). By similar methods glycine was shown to block reabsorption of arginine, but competition was not demonstrated between arginine and creatine. He has proposed a common renal mechanism for tubular reabsorption of the five substances. The results are not entirely conclusive because extremely high rates of filtration and secretion of creatine were employed. Deductions were thus based on changes that are close to the limits of the methods in the relatively small amounts of creatine reabsorbed. In addition, Beyer (20) has pointed out the drawbacks in such studies of using non-specific methods for the determination of amino acids. If the substances do share a common reabsorptive mechanism, it would be expected from the present studies that the amino acids would block GAA reabsorption. Evidence is as yet lacking on this point, except for the observation of Borsook (21) that GAA excretion increases following the ingestion of glycine or arginine or both, with a fall in place of the rise in serum GAA concentration that would be expected, if the increased excretion were solely the result of increased synthesis.

Thyroxine and desoxycorticosterone acetate have recently been shown to reduce the maximal tubular reabsorption of creatine in hypothyroid subjects (22). Since GAA is normally reabsorbed with less facility than creatine, similar changes in its excretion might be expected from these agents. Bodansky (23) found high levels of excretion of GAA in hyperthyroidism, but from the values tabulated above, it seems likely that this effect was secondary to creatinuria. That the increase in GAA excretion seen in man during ingestion of methyl testosterone does reflect increased synthesis is evident, first, from isotope studies (24) and, secondly, from the fact that the serum level of GAA rises before that of creatine (25).

The mechanism of the renal tubular reabsorption of creatine and of GAA is obscure. *In vitro* phosphorylation of added creatine with rat kidney homogenates has been demonstrated by Potter (26) and it is possible that creatine is phosphorylated by the tubular cell on reabsorption. But no such evidence exists to suggest that glyocyamine phosphate may be formed during reabsorption of guanidoacetic acid. Experiments are in progress to determine the effect of compounds other than creatine on this reabsorptive system.

In view of the magnitude of the increase in GAA excretion following the induction of a slight creatinuria, it is apparent that GAA tolerance tests must be controlled by simultaneous measurement of its renal excretion. Likewise, it is apparent that the relative or absolute level of GAA excretion cannot be taken as an index of the rate of its endogenous synthesis or of its methylation.

SUMMARY

In normal males it was found that elevation of serum creatine within physiological limits increased the excretion of guanidoacetic acid and reduced its renal tubular reabsorption, both at endogenous and at elevated serum levels. The reverse effect could not be conclusively demonstrated. Increases in renal excretion of guanidoacetic acid may be a reflection of creatinuria rather than of change in the rate of guanidoacetic acid synthesis or methylation.

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RÔLE OF THE KIDNEY IN PATHOGENESIS OF HYPERTENSION AS DETERMINED BY A STUDY OF THE EFFECTS OF BILATERAL NEPHRECTOMY AND OTHER EXPERIMENTAL PROCEDURES ON THE BLOOD PRESSURE OF THE DOG

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ONE of the difficulties involved in determining experimentally the exact rôle which the kidney plays in the pathogenesis of hypertension has been the impossibility of ablating this organ without introducing the fatal effects of interfering with its normal excretory function. For this reason, it has been impossible to apply the simple experimental procedure of extirpation which has aided so much in the elucidation of the function of other organs. It is, in fact, generally assumed that bilateral nephrectomy causes no change in the blood pressure. However, animals subjected to this procedure in the past have survived for such relatively short periods and were in such poor condition that any conclusion as to the effect of nephrectomy on the blood pressure based on observations of such animals is not convincing.

Indirect studies designed to elucidate this problem, such as the effect of unilateral or bilateral nephrectomy in the hypertensive animal (1) or in the parabiotic rat (2), have indicated that nephrectomy results in an elevation in blood pressure and that the presence of intact kidneys is essential for maintaining the normotensive state (3). In the present study, it has been possible to maintain bilaterally nephrectomized dogs in a state of good health for a longer period than has hitherto been possible. To determine the effect of the presence of renal tissue incapable of exerting any excretory function on the blood pressure, animals with one ureter ligated or implanted into the small intestine or abdominal vena cava and the contralateral kidney removed and those with bilaterally ligated ureters were studied. Observations on the blood pressure in these animals permit one to draw certain definite conclusions as to the rôle of the kidney in the pathogenesis of hypertension and indicate that the kidney, aside from its excretory function, is responsible for the maintenance of a normal blood-pressure level.

METHODS

Adult mongrel dogs of either sex were used throughout this study. Nephrectomy was performed under ether anesthesia either in two stages with an interval of a week or more between the two operations or at one operation. In the former case, a lumbar approach was used; in the latter, the abdominal transperitoneal route was utilized. The former procedure involves much less operative shock and is preferred if adequate facilities are available for housing the animals in the interval between the operations.

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In another group of dogs, the right ureter was freed from its accompanying blood vessels, ligated in close proximity to its entrance into the bladder, and cut. The ligated end proximal to the bladder was buried by fine sutures into the serosal lining of the bladder. The open end was transfixed with three sutures which were then passed through an opening into the small bowel and an uretero-intestinal anastomosis performed by the usual surgical procedures. This is essentially the Coffey operation as performed on the human subject² except for the fact that the implantation is made into the small intestine from which the urine is resorbed.

In a comparable series of animals the right ureter was implanted into the abdominal vena cava and the left kidney ablated. Approach to the vena cava was either through a circular incision across the upper abdominal wall or preferably through a right rectus incision. After exposing the vena cava a segment was isolated between clamps, the ureter prepared as described in the preceding paragraph, and anastomosed to an opening made in the vena cava. After an interval of one to 3 weeks to allow union at the site of the anastomosis, the contralateral kidney was removed through a lumbar incision.

In the fourth series of animals, both ureters were doubly ligated and divided or the right ureter ligated and divided, and after a period of at least two weeks, the left kidney was ablated.

Mean blood pressures were determined by direct puncture of the femoral artery with a No. 18-gauge needle attached through a 3-way valve to a syringe and to a mercury manometer (1).

Two procedures have been utilized for abolishing uremia and prolonging life of the animals prepared as described above. Prolongation of life for several days beyond that normally observed in the absence of renal excretory function was made possible by feeding the animals a diet practically free of electrolytes. Such a diet was prepared either by dialysis of the animals' normal food or by feeding a mixture of casein with a very low sodium content³ to which was added glucose and lard. Animals maintained on such diets remain in much better health following bilateral nephrectomy and survive longer than do those on a free diet. We have observed that the intensity of many of the symptoms observed in the nephrectomized animal, as well as in the human patient dying in uremia, is apparently due to the accumulation of ingested electrolytes (particularly potassium) for it is greatly mitigated if electrolytes are excluded from the diet.

The survival period of the nephrectomized dog varies usually between 2 and 7 days, depending on the original state of health of the animal, the operative procedure, conditions under which the animal is kept etc. Under the conditions of our experiments, survival extended from 2 to 5 days. However, when the animals were given a 'salt-free' diet, as described above, survival was extended to at least 5 days and usually longer. To maintain animals with no renal function beyond this time required the application of an 'artificial kidney' to remove the accumulated urea and

² We are indebted to Dr. Carl Moyer for demonstrating this operation to us and performing it on *dog 73*.

³ Liberal supplies of this casein were supplied by Mead-Johnson and Company through the courtesy of Dr. Charles Bills.

other waste products from the body and to maintain normal water, electrolyte and acid-base equilibria.

The use of the artificial kidney has been described elsewhere (4). Its successful application made it possible to prolong the life of bilaterally nephrectomized dogs or dogs otherwise deprived of renal excretory function for periods sufficiently long to permit one to draw definite conclusions as to the rôle of the kidney in the pathogenesis of hypertension. It was necessary to apply the artificial kidney on the 5th or 6th day following exclusion of renal excretory function and at intervals of 3 or 4 days thereafter. The femoral artery was ligated and the blood, after passing through the 'artificial kidney', returned to the femoral vein. A period of dialysis of $2\frac{1}{2}$ to 3 hours was usually required to lower the urea content of the blood from 470 to 600 to less than 100 mg. per cent.

RESULTS

Effect of Bilateral Nephrectomy. In table 1 are given the mean blood pressures of 38 dogs following bilateral nephrectomy. It is evident from the results that following removal of the kidneys there is a tendency for the blood pressure to rise. This rise is evident on the third day following nephrectomy and increases thereafter as long as the animal survives. In ten instances (dogs 35, 39, 54, 70, 75, 76, 84, 86, 89, 93, and 96) values of 150 mm. Hg, which is usually taken as definite evidence of hypertension in the dog, were not attained. However, even in the case of these animals there is in every case but one (dog 35) a tendency for the blood pressure to rise above the preoperative level. The average mean blood pressure as seen in the last line of of table 1 rises gradually over the preoperative level as survival is extended.

Microscopic study of the tissues of the nephrectomized animals revealed (5) widespread cardiac damage—subendocardial hemorrhages, myocardial necrosis etc.—which would tend to prevent a sustained maximal rise in blood pressure. This is comparable to the decline in blood pressure observed in human hypertensives suffering from cardiac failure. The changes in the heart account probably for the observed rises being less pronounced than they might otherwise be.

The general trend of the blood pressure rise in the nephrectomized dog is shown graphically in figure 1 in which data on 5 dogs are reproduced to show the gradual rise and extent of the blood pressure rise which follows nephrectomy.

Effect of Bilateral Ureteral Ligation. In table 2 are recorded the blood pressure responses of 12 dogs in which both ureters were ligated and divided, the kidney being left intact. This operation, in accord with the findings of earlier observers (6-10), results in a rise in blood pressure which is sometimes evident on the day following the ligation and reached its maximum (with the single exception of dog 23) on the fourth day, declining thereafter to the normal level. This secondary decline has been overlooked by previous workers since their animals failed to survive for a period sufficiently long for it to manifest itself.

Ligation of the ureters results in hydronephrosis and marked changes in the kidneys as well as in a period of survival which is less than that observed following nephrectomy. The observed rise in blood pressure is apparently due to the liberation of a pressor agent from the injured kidney for a similar rise is noted even when only

TABLE 1. EFFECT OF BILATERAL NEPHRECTOMY ON THE MEAN BLOOD PRESSURE OF THE DOG

DOG	PREOPERA- TIVELY	DAYS FOLLOWING FINAL NEPHRECTOMY											
		1	2	3	4	5	6	7	8	9	10	14	17
26	110		145		160								
57	120	130		140	150								
66	115	120	135	155	160								
89	110	120	130	130	100								
9	110		120		150	160							
39	105	100		125		130							
54	110	110	135		120	145							
62	100			140	145↑	160							
68	100	110		150		170							
70	100		120		140	125							
78	100	100		140		160							
93	100	100	115	125	125	120							
135	110			140		160							
15	110			150		150	150						
19	120	110	120	120	150	140	155						
44	105	120	125	135	140	155	160						
47	120	130	130	135	150	120	160						
64	100	100		130	125	140↑	150						
71	100	105	115		140	155	175						
75	100	100	110		130		135						
79	110	110	115	135	155	170	180						
86	100	110	115	110	125	130↑	120						
24	110	100	110	120	140	150	170↑	170					
29	110	120	140	150		195	180↑	160					
34	110			130		160	160↑	140					
65	105		110	140	150	180	180↑	140					
76	110		115		130	140	135	140					
84	125	125	130	135	120	130↑	135	120					
96	100		110	115	130	130↑		135					
97	115		125	140	150	160↑		140					
14	120	110			150↑	185↑	175	170	180				
22	120			120	135	150	145↑	130	185				
61	80	80	90	100	110	150↑		130	140				
32	115			125		180	150↑		170	170			
33	120			130		160	150↑	160	140	160			
35	110	100	115	130		120	100↑	105	115				
40	120			160		170	180↑	170	190↑	200	195		
73	100		110		120	130	130↑	125	130↑	125	↑	160	170
Average elevation over preoperative level in mm. Hg.		2	13	24	29	44	43	32	44	41	75	60	70

Arrows indicate the animal was attached to the 'artificial kidney' on that day. Mean blood pressures in mm. Hg.

one ureter is ligated as shown in table 3 and fails to occur if one implants the ureter into the small intestine or vena cava as shown later. As seen in table 3, there is a rise in blood pressure following ligation of one ureter, the contralateral kidney and ureter

being left intact. Except in *dog 88*, which remained hypertensive during the 3 months it survived the operation, the blood pressure in the other animals returned to its normal preoperative level and remained so. In the case of *dog 88*, we are presumably dealing with the occasional animal in which unilateral nephrectomy, unilateral application of a figure-of-eight ligature, or other interference with renal

Fig. 1. MEAN BLOOD PRESSURE in mm. Hg of 5 dogs following bilateral nephrectomy. The animals were attached to the 'artificial kidney' for a period of 3 hours on the 5th day following nephrectomy.

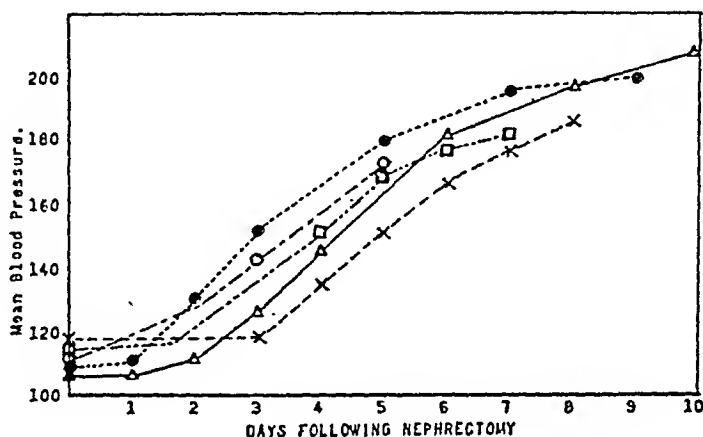


TABLE 2. EFFECT OF LIGATING AND DIVIDING BOTH URETERS ON THE MEAN BLOOD PRESSURE OF THE DOG

DOG	PREOPERA- TIVELY	DAYS FOLLOWING OPERATION									
		1	2	3	4	5	6	7	8	9	12
38	120	155	170								
51	105	120	145	150							
37	120		150	160	155						
49	120	120	140	145	180						
41	110		125	150	175	150					
27	110		110		230	145↑	120				
43	110	120	130	135	150	110↑	110				
45	115	110	140	170	150	130	110				
46	120	120	150	145	160	130↑	110	95			
23	120			160	135↑	130	160	200	190		
31	115			155	175	150↑	125	130	120↑	120	
100	120				120	110↑		100	100	110↑	120

Arrows indicate time when animal was attached to 'artificial kidney' for a period of 2 to 3 hours. Mean blood pressures in mm. Hg.

function as by ligation of the ureter suffices to induce permanent hypertension without removing the contralateral kidney as is usually necessary (1, 11).

Bilateral ureteral ligation not only stops the excretory activity of the kidney but also interferes with its blood supply because of pressure on the renal pelvis. There is congestion of the kidney, hydronephrosis and often perirenal hemorrhage. Nevertheless, certain activities of the kidney are apparently still preserved, for example, its glycogenic function (12) and presumably its capacity to maintain a normal blood pressure after subsidence of the acute rise which follows this operation.

In order to avoid the changes induced by ureteral ligation, the following observations in which the ureter was implanted into the gut or vena cava were performed.

Effect of Implanting the Right Ureter in Small Intestine and Ablating Left Kidney. As a control for the experiments cited in which both kidneys were removed, the right ureter was implanted into the duodenum or jejunum and several weeks later the left kidney was removed. The blood pressures of such animals as shown in Table 4 does not become elevated when the animals survive (by the aid of the artificial kidney), as in the case of dog 77, for as long as a month. The blood urea levels of such animals rise essentially to the same levels as those observed in bilaterally nephrectomized dogs as is evident from the data of table 5.

TABLE 3. EFFECT OF LIGATING AND DIVIDING ONE URETER, THE OTHER KIDNEY AND URETER REMAINING INTACT, ON THE BLOOD PRESSURE OF THE DOG

DOG	PREOPERATIVE LEVEL	DAYS FOLLOWING OPERATION									
		1	2	3	4	5	6	7	8	9	10
90	120	120	130	140	150	165	160	150	140	135	120
85	90	100	105	110	125	90	90	90	85	90	90
83	110	110	120	140	155	150	140	120	110	110	110
88	115	135	135	140	130	125	140	145	150	145	150
91	115	135	130	135	125	145	140	135	130	120	110
92	105	115	130	135	125	110	115	110	110	105	100

Mean blood pressure in mm. Hg.

TABLE 4. EFFECT OF IMPLANTING RIGHT URETER INTO THE SMALL INTESTINE AND REMOVING LEFT KIDNEY ON MEAN BLOOD PRESSURE OF THE DOG

DOG	PRIOR TO NEPHREC- TOMY	DAYS FOLLOWING NEPHRECTOMY													
		1	2	3	4	5	6	7	8	9	10	11	12	15	30
121	110	110		100	110	100									
124	120				135	150									
126	110	110		110	120				110	100		90			
123	105	110	110	100	100	90↑	70	80	80	↑	85	90	80		
74	105	100	105	110	110	90	100↑	110	110↑	100	80	90↑	90	90	
77	110	110	110		115	115	110↑	110	110	110	110↑	100	95	95	90

Arrows indicate application of artificial kidney. Mean blood pressures in mm. Hg.

Despite the rapid rise in non-protein-nitrogen of the blood which follows the implantation of a ureter into the duodenum and removal of the contralateral kidney, it may be objected that such preparations are not comparable to the bilaterally nephrectomized animal since some constituents other than the non-protein-nitrogenous bodies may not be reabsorbed from the intestine. Such an assumption is compatible with the observations of previous investigators who have noted that the degree of uremia and the period of survival of such animals is dependent upon the level at which the anastomosis is made. If made high in the duodenum, complete reabsorption of the urine occurs, there is a rapid increase in the non-protein-nitrogen level of the blood, and the animals survive for periods comparable to that following nephrectomy (13). On the other hand, if the transplant is made lower in the ileum,

there is no increase in the creatinine, creatine, uric acid or amino acid content of the blood and the animals survive for periods of 14 to 20 days (14).

Effect of Implanting the Right Ureter in Vena Cava and Removing the Left Kidney.
In order to obviate the objections that the failure of the blood pressure to rise in dogs with a ureteral implant into the duodenum is due to the excretion by the bowel of certain catabolites, the series of experiments cited in table 6 were performed in which the ureter was implanted into the abdominal vena cava. Under these conditions no excretion is possible while relatively normal renal tissue remains in the organism.

TABLE 5. EFFECT OF IMPLANTING RIGHT URETER INTO THE SMALL INTESTINE AND REMOVING LEFT KIDNEY ON THE MEAN BLOOD PRESSURE AND BLOOD UREA LEVELS OF TWO DOGS

	DOG 74		DOG 77	
	Mean blood pressure	Blood urea level	Mean blood pressure	Blood urea level
	mm. Hg	mg. %	mm. Hg	mg. %
Prior to removing left kidney.....	105	30	110	35
Days following nephrectomy				
1.....	100		110	
2.....	105	248	110	
3.....	110		110	320
4.....	110	415	115	
5.....	90	475	115	512
6.....	100		110	517
7.....	110	389	110	
8.....	110		110	428
9.....	100		110	
10.....	80	537	100	343
11.....	90		95	
12.....	90	520	100	471
13.....			110	514
15.....	90	600		
16.....			115	610
21.....			95	574
25.....			95	450
30.....			90	612

Arrows indicate days on which animals were attached to the artificial kidney.

As noted in table 6, the blood pressure under these conditions remains at its normal level.

DISCUSSION

In figure 2 are illustrated graphically the effects on the blood pressure of the various manipulations on the kidney carried out in the present investigation. The curves were constructed by averaging the differences between the blood pressure on each day following operation and the pre-operative levels. It is evident from figure 2 and the data of tables 1 to 6 that bilateral nephrectomy results in hypertensive blood pressure levels in the dog, and that in the presence of renal tissue in the body, as in the uretero-intestinal and ureterovenous anastomoses, despite the absence of

excretory function, the blood pressure remains at its normal level. When, however, the ureters are ligated there is an acute rise in blood pressure which returns to normal at a time when the blood pressure in the nephrectomized animal is still rising. The latter rise is generally accepted as being due to the liberation of a pressor agent (angiotonin or hypertensin) from ischaemic renal tissue. If only one ureter is ligated, this transient rise in blood pressure also appears and if the contralateral kidney is removed after the blood pressure returns to normal, no further elevation in blood pressure occurs.

TABLE 6. EFFECT OF IMPLANTING RIGHT URETER INTO VENA CAVA AND REMOVING LEFT KIDNEY ON THE MEAN BLOOD PRESSURE OF THE DOG

DOG	PRIOR TO NEPHRECTOMY	DAYS FOLLOWING NEPHRECTOMY											
		1	2	3	4	5	6	7	8	9	10	11	12
II7	115		100	110	100								
II2	130		120		130	135							
II21	110	110		110	110	100							
II8	110		120		140		120						
II5	110	110		110	110		105	110					
II3	115		120		130		130		135				
IO8	110	120		130		150	140		130	120			
IO4	110		100		100	75	110			135	130	125	110

Arrows indicate application of artificial kidney.

Mean blood pressures in mm. Hg.

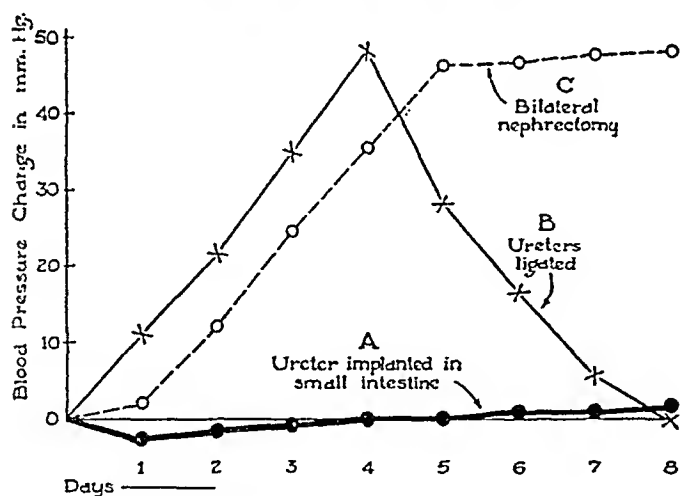


Fig. 2. AVERAGE CHANGES IN BLOOD PRESSURE of three series of 6 dogs; A, with the right ureter implanted into the small intestine and the left kidney removed (●—●); B, with both ureters ligated (x—x); and C, with both kidneys removed (O—O). Note the constancy of the blood pressure in A, the transient rise with a return to normal levels in B and the continuous sustained rise in C. The ordinates indicate the changes in blood pressure over the preoperative levels; the abscissae, days following nephrectomy or ligation of the ureters.

Previous investigators have also noted the maintenance of a normal blood pressure level following the anastomoses of the ureters and the ileum (15) or the venous circulation (9, 15) and following bilateral occlusion of the renal veins (16). However, in these experiments the animals survived for only 3 days or less when all excretory function was abolished. Consequently, they are of little significance since one may justifiably attribute the observed results to the poor state of the animals or a failure of sufficient time to elapse for hypertension to manifest itself.

The rise in blood pressure to hypertensive levels of the nephrectomized dog obviously cannot have been due to the liberation of renin (hypertensin) or other pressor agents derived from the kidney since no renal tissue was present in the animal. Nor

can it be argued that the rise in pressure was due to any interference with normal hemodynamics by the use of the artificial kidney since the rise in pressure was evident even before the animal was subjected to this manipulation, and no rise occurred when uremic animals with renal tissue present were treated similarly.

The failure of previous investigators (17) to be impressed by the rise in blood pressure induced by nephrectomy must be attributed to the poor condition of their animals, as evidenced by the short period of their survival. As a matter of fact, perusal of the literature reveals instances of hypertensive levels in dogs following nephrectomy, as, for example, in the papers of Wintermitz, *et al.* (18), and Harrison, *et al.* (8) in whose series of nephrectomized animals several instances of definite hypertension occurred. However, since these rises occurred only occasionally, they were disregarded by these authors.

The present experiments offer direct evidence for the view that the kidneys exert an action other than their excretory function in the organism (19, 20). Not only is this demonstrated by the difference in the observed blood pressures in the presence and absence of renal tissue but also in the fate of the animals under the two conditions. The bilaterally nephrectomized animals, despite the removal of the excretory products by the 'artificial kidney', developed those pathological changes in the tissues which are associated with so-called malignant hypertension. These occasionally included hemorrhages in the eye-grounds and changes in the arterioles, skeletal muscles, myocardium and smooth muscle generally. Intussusception of the intestine which is common in dogs in which malignant hypertension is induced by the application of a clamp to one renal artery and removal of the opposite kidney occurred in over 10 per cent of our nephrectomized dogs surviving 8 days or more. A detailed pathological report of these studies and their implications will be reported elsewhere (5). They offer material evidence for determining the relative rôle played by the elevation in blood pressure, the accumulation of waste products and the presence or absence of renal tissue in inducing the pathologic changes observed in malignant hypertension.

Geer and Dragstedt (15) concluded from their observations on 4 dogs that the deviation of urine from one kidney into the blood stream produces no toxic symptoms. However, one of their 4 animals died on the 13th day while the others were killed on the 11th, 28, and 37th day. In our own series, 4 dogs in which the contralateral kidney was not removed died on the 7th, 12th, 13, and 30th days. It would appear therefore, that diversion of the urine into the blood stream is not entirely innocuous as appears also from our pathological studies of the tissues of these animals.

It may be objected that under the experimental conditions in which renal tissue is present without elevation in the blood pressure, the blood pressure fails to rise because of the detrimental effects of the experimental procedures. However, in cases of the uretero-intestinal anastomoses, the lesions present were much less pronounced than in those of bilaterally-nephrectomized dogs which in comparable periods of time manifest definite hypertension. In cases of the uretero-venous anastomoses, also, survival extended well beyond the time that elevations in blood pressure appear in the bilaterally nephrectomized animals. Our data also show that there is no close correlation between the elevation of the blood pressure and the severity of the lesions seen at autopsy.

SUMMARY

The effect of the following procedures on the mean blood pressure of the dog was determined: 1) bilateral nephrectomy; 2) bilateral ligation of both ureters; 3) ligation of one ureter, the other remaining intact; 4) implantation of one ureter into the small bowel with removal of the contralateral kidney; and 5) implantation of one ureter into the abdominal vena cava with removal of the contralateral kidney. The period of survival of the animals deprived of renal excretory function was prolonged by maintaining them on an electrolyte-free diet and by application of an artificial kidney at intervals of 4 or 5 days.

The blood pressure of the nephrectomized animals gradually rose to hypertensive levels and at autopsy revealed the pathological findings of malignant hypertension. Ligation of the ureters resulted only in a temporary rise in blood pressure. If the contralateral kidney was removed following the return of the blood pressure to normal, no elevation in blood pressure occurred. Likewise, in animals with one ureter implanted in the small intestine or into the vena cava and the contralateral kidney removed, no hypertension resulted.

It is concluded that nephrectomy results in hypertension and that the presence of intact renal tissue is essential for the maintenance of the normotensive state. The present studies offer direct evidence for the view that the kidney normally, in addition to its excretory function, also exerts a function which is concerned in the maintenance of normal blood pressure levels. Hypertension of renal origin according to this view is not due to the liberation of a pressor agent, but results from a failure of this activity of the kidney.

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EFFECTS OF EMOTIONAL DISTURBANCE ON WATER DIURESIS AND RENAL BLOOD FLOW IN THE RABBIT¹

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IN THE dog and man under normal conditions, variations in urine flow are to a great extent independent of the glomerular filtration rate, water diuresis being effected primarily by decreased tubular reabsorption (1). In rabbits, however, Kaplan and Smith (2) reported that filtration rate and urine flow varied in a parallel manner, water diuresis being accompanied by an increase in the filtration rate. These observations, which have been confirmed by Dicker and Heller (3) and Forster and Maes (4), place the rabbit in an anomalous position among the mammals so far investigated. Our reinvestigation of this problem was occasioned by the extreme difficulty which we encountered in some experiments in producing water diuresis in certain rabbits, and further study has led us to the conclusions that the apparent relationship between filtration rate and urine flow in the rabbit is physiologically fortuitous, in that when present it is attributable to a reversible reduction in filtration rate, associated with renal ischemia reflexly induced by the manipulation of excitable animals; as the renal and glomerular circulation recover, the urine flow increases *pari passu* with the filtration rate. In the absence of renal ischemia, variations in urine flow appear to be mediated independently of the filtration rate, as in man and the dog.

METHODS

Simple water diuresis experiments were performed on 42 unanesthetized adult male rabbits weighing from 2300 to 4400 gm. The animals were maintained on a diet of dry oats and dried greens and had free access to water. Depending upon the conditions of the experiment, they were either kept in a closed box or tied to an animal board throughout the period of observation. For the induction of water diuresis they were given 50 cc. distilled water per kg. body weight by stomach tube. Urine was obtained through an indwelling no. 16 French Foley catheter with a 5-cc. bag in the bladder inflated with water to insure against expulsion of the catheter. After each collection period the bladder was washed with 5 cc. of distilled water and 10 cc. of air. The efficiency of this method of bladder emptying was confirmed at numerous post-mortem examinations which failed to reveal any residual bladder urine.

The filtration rate was measured by the exogenous creatinine clearance (2), and the effective renal plasma flow by PAH (p-aminohippuric acid) clearance (5). Priming and maintenance doses of these substances were calculated to maintain plasma levels of 30 mg. per cent for creatinine and 2 mg. per cent for PAH. The sustaining dose was given as a constant intravenous infusion in isotonic saline into the marginal vein of the ear at a rate of 0.3 cc. per minute by means of a mercury gravity

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drip pump. The priming dose was injected directly into the rubber tubing feeding the sustaining infusion. One hour was allowed to elapse from the time of the priming dose before starting the first urine-collection period in order to insure equilibrium levels of the test substances throughout the body fluids. Urine-collection periods varied from 10 to 60 minutes in length, depending upon the urine flow. Blood samples were obtained by means of cardiac puncture. Blood pressure measurements were recorded by the method of Grant and Rothschild (6). Creatinine was determined by the standard Folin (7) method and PAH by the technique of Smith *et al.* (5). Emotional disturbances were created by faradic shocks applied to the bare skin of the thorax; by loud, banging noises; by pinching; and, in hyperexcitable animals, by merely keeping them tied to an animal board in the supine position. When freedom from emotional stress was desired the rabbit was placed upright in an enclosed box, allowing free egress of the head, and disturbing sounds were avoided.

RESULTS

Effect of Emotional Stress on Water Diuresis. Water diuresis was induced in a group of hyper-excitable rabbits when secured to an animal board in the supine position, and compared with the diuresis obtained in the undisturbed condition. This stimulus was sufficient to produce obvious tenseness and hyper-irritability for 5 hours, with the exception of *rabbit 10*, table 1, which was conspicuous by its docility. The results of 13 water-diuresis experiments on the animal board and in the apparently non-disturbing environment of the box are contrasted in table 1 and figure 1. The difference in diuretic response is very marked. The average urine output in a 5-hour period of the 7 animals on the board was 24.8 per cent of the total water ingested, as compared to 87.1 per cent for the animals in the box. The good diuretic response obtained with *rabbit 10* on the board seemed to be related to his apathy or docility.

The supine position alone does not seem to affect water diuresis, since the same degree of inhibition of diuresis was obtained when the animals were tied to the board in the prone position. Forster and Maes (4) also noted that variations of the horizontal position resulted in little change in the renal function of rabbits.

Effect of Emotional Stress and Painful Stimuli upon Renal Blood Flow. Water diuresis was induced by placing the rabbits in the box after water administration, and at the height of the diuresis the animals were disturbed by unpleasant stimuli. The results of 15 such experiments are recorded in table 2, and the course of a typical experiment is shown in figure 2. In all cases the disturbing stimuli were immediately followed by a marked decrease in urine flow, effective renal plasma flow and filtration rate, and a more gradual increase in the creatinine U/P ratio, representing increased tubular reabsorption of water. There was no consistent change in the filtration fraction; in 8 instances it was elevated, in 4 it was decreased, and in 3 there was no significant change. The blood pressure, if it changed at all, increased by 5 to 20 mm. Hg following the stimulus. Oliguria was frequently very severe, lasting from 30 to 120 minutes. The experiment was terminated in 6 cases by death of the animal in convulsions, apparently because of water intoxication resulting from prolonged oliguria in the face of excessive hydration coupled with prolonged intravenous infusion at the rate of 0.3 cc. per minute. These convulsive deaths were similar to those observed by Rowntree (8) in his study of water intoxication. Post-mortem examination revealed only distention of the venae cavae, and in only one instance was pulmonary edema present. Although a few of the animals tested were relatively

resistant to excitation, oliguria could always be provoked by increasing the strength of the stimulus.

From these data it appears that the oliguria associated with painful stimuli and emotional stress in the rabbit is caused primarily by a reduction in renal blood flow and filtration rate and to only a minor degree by increased tubular reabsorption of water.

TABLE 1. EFFECT OF EMOTIONAL STRESS UPON WATER DIURESIS

RABBIT NO.	PER CENT OF INGESTED FLUID EXCRETED On Board	PER CENT OF INGESTED FLUID EXCRETED IN 5 HOURS In Box
2	16.6	102
3	21.4	106
6	7.00	
7	4.80	
8		70.0
9	11.5	63.0
10	83.0	
11	29.6	102
14		79.5
Average	24.8	87.1

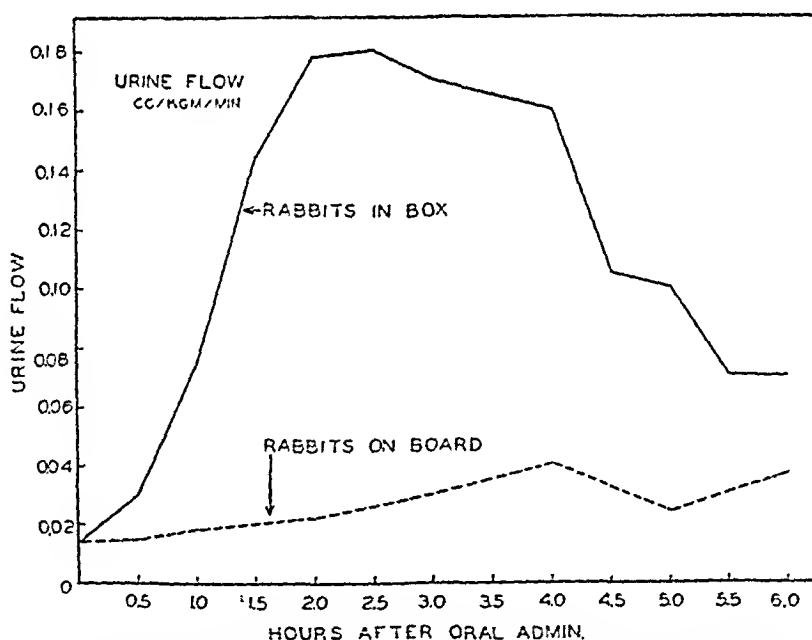


Fig. 1. COMPARISON OF AVERAGE WATER DIURESIS CURVES obtained with excited and quiescent rabbits, revealing the inhibition of water diuresis in the disturbed animals.

Relationship between Glomerular Filtration Rate and Urine Flow in the Unexcited Rabbit. Animals which appeared to us to be least disturbed by handling were chosen for this series of experiments, and the environment was maintained free of disturbing stimuli. The results of 8 such experiments are tabulated in table 3 and figure 3. The data show that with such selected animals and under suitable conditions it is possible for the urine flow to vary as much as 16-fold during water diuresis

without significant changes in the filtration rate. The normal variation in urine flow in the unexcited rabbit appears to be a function of tubular reabsorption of water, as in the other common mammals.

Factors Controlling Effect of Emotional Stress and Painful Stimuli on Renal Blood Flow. Three possible mechanisms were considered: 1) general circulatory effects; 2) stimulation of renal vasomotor apparatus by neural transmission; 3) stimulation of renal vasomotor apparatus by humoral factors.

TABLE 2. EFFECT OF EMOTIONAL STRESS AND PAINFUL STIMULI ON RENAL CIRCULATION

RAB- BIT NO.	QUIET PERIOD					PERIOD OF EMOTIONAL STRESS					RATIO OF FUNCTIONS BEFORE AND AFTER				
	Urine Flow Be- fore	Stim- ulus	Creat. Clear.	PAH Clear.	Fil- tra- tion U/P Ratio	Type of Stim- ulus	Urine Flow Follow- ing	Stim- ulus	Creat. Clear.	PAH Clear.	Fil- tra- tion U/P Ratio	Urine Flow	Creat. Clear.	PAH Clear.	Fil- tra- tion U/P Ratio
	cc/ min.	cc/ min.	cc/ min.				cc/ min.	cc/ min.	cc/ min.			cc/ min.	cc/ min.	cc/ min.	
10 ¹	1.40	7.75	45.5	5.55	0.170	on board	0.252	2.34	12.1	9.3	0.193	5.55	3.31	3.77	0.598
3 ¹	0.706	9.52	40.1	13.6	0.237	supine faradic shock	0.067	2.21	10.2	33.0	0.217	10.5	4.31	3.93	0.412
13 ¹	0.939	7.50	27.4	8.00	0.274	on board prone heart	0.037	0.637	2.53	17.3	0.252	25.4	11.8	10.8	0.462
11 ¹	0.146	7.88	89.0	54.0	0.089	puncture heart	0.064	2.04	21.5	33.4	0.095	2.28	3.86	4.14	1.62
17 ¹	0.900	15.2	199.0	21.7	0.078	puncture escape	0.28	9.25	12.2	33.0	0.076	3.21	1.64	1.63	0.658
25	1.50	14.4	85.7	9.60	0.168	reaction faradic	0.59	7.93	21.5	13.3	0.368	2.54	1.82	3.98	0.794
25	0.590	7.93	21.5	13.3	0.368	shock faradic	0.063	2.96	6.06	47.2	0.488	9.37	2.68	3.55	0.284
22	0.606	11.6	38.1	19.1	0.305	shock heart	0.300	8.61	14.7	28.7	0.585	2.02	1.35	2.59	0.666
22	0.426	11.7	29.9	27.4	0.293	puncture tube	0.076	3.09	11.05	40.6	0.280	5.61	3.79	3.62	0.675
155	0.222	11.1	66.4	52.0	0.168	feeding tube	0.109	6.70	29.2	61.5	0.230	2.04	1.66	2.28	0.846
158	0.1320	11.9	82.0	90.0	0.145	feeding on board	0.059	7.16	39.4	121.3	0.181	2.24	1.66	2.08	0.741
164	0.191	8.80	27.4	46.0	0.321	supine tube	0.037	3.03	10.4	83.3	0.298	5.16	2.86	2.64	0.552
157 ¹	0.299	6.30	24.1	21.0	0.261	feeding tube	0.145	3.59	18.4	24.8	0.195	2.06	1.75	1.31	0.844
150	0.132	11.9	82.0	90.0	0.145	feeding heart	0.059	7.16	39.4	12.1	0.181	2.24	1.66	2.08	0.744
150	0.934	7.78	43.8	8.33	0.178	puncture	0.071	4.56	20.3	6.4	0.225	13.1	1.71	2.16	0.130

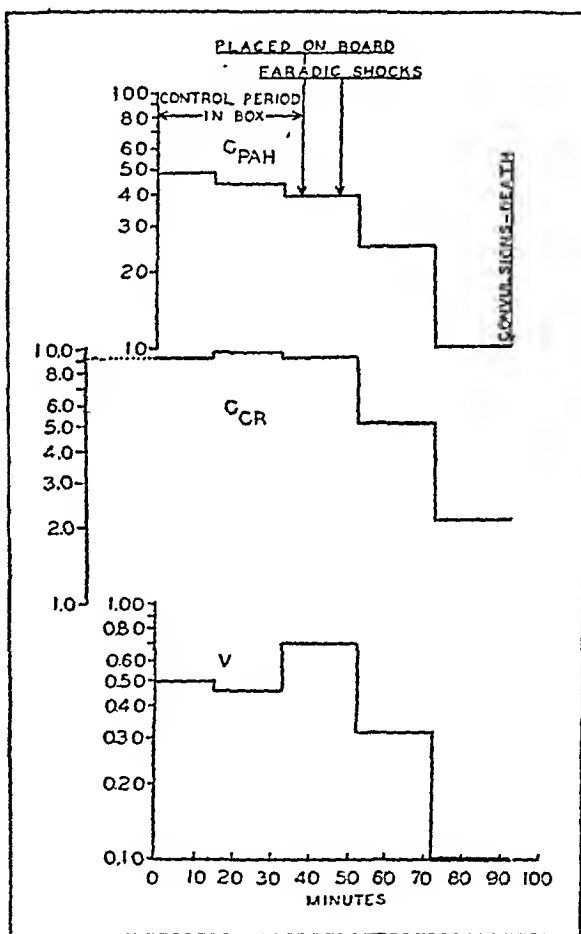
¹ These animals died with convulsions, apparently due to water intoxication.

1) Since the blood pressure of all the animals tested remained either constant or revealed a moderate rise, the depressed renal blood flow could not be attributed to decreased blood pressure in any instance. 2) In order to investigate the effect of neural impulses transmitted via renal nerves, we repeated the excitation experiments in 5 rabbits with denervated kidneys. Both kidneys were denervated by stripping all visible strands of tissue from the hilar vessels and ureters, followed by local application of 5 per cent phenol for 2 minutes. The experiments were performed from 10 to 14 days post-operatively. The results of these experiments duplicate those in

unoperated animals: painful stimuli and emotional excitement caused a marked reduction in diuresis, filtration rate and plasma flow. Our results indicate that the renal nerves are not necessary for the production of renal ischemia.

3) Several experiments were designed to investigate the influence of administered antidiuretic hormone and adrenalin in the production of oliguria. At the height of water diuresis under quiescent conditions, 2 milliunits of pitressin were administered intravenously to 4 rabbits weighing about 2500 gm. This dose was sufficient to produce antidiuresis in 3 of the 4 animals, with only slight and transitory decreases in the renal plasma flow and filtration rate in 2 of the 3. The inhibition of diuresis

Fig. 2. COURSE OF A TYPICAL EXPERIMENT in which faradic shocks were applied at the peak of water diuresis, marked parallel depression of renal blood flow, glomerular filtration and urine flow results.



was due primarily to increased tubular reabsorption of water. The respective changes in the creatinine U/P ratios were 26 to 73, 28.5 to 64.8 and 14.8 to 45.0. In contrast, 400 γ of epinephrine caused a marked reduction in renal plasma flow and filtration rate and marked oliguria, with little effect upon the creatinine U/P ratio. In one rabbit the urine flow decreased from 0.162 to 0.065 cc/min., the renal plasma flow from 33.9 to 5.92 cc/min., and the filtration rate from 5.20 to 1.71 cc/min., with the creatinine U/P ratio changing from 32.1 to 26.4. Epinephrine in doses of from 50 to 120 γ failed to produce consistent effects. It appears that the oliguric state stimulates the renal effects of relatively large doses of epinephrine. Dibenamine (9) was administered to 7 animals at the height of oliguria to determine whether this

adrenolytic agent had any effect upon the mechanism. The drug was given intravenously in doses of 5 mg/kg. of body weight. In 3 rabbits with intact renal nerves this drug had no significant effect. In 3 of the 4 animals with denervated kidneys there was a definite increase in the renal plasma flow and filtration rate immediately following its administration, with no change in the 4th. The effect of dibenamine upon the action of large doses of adrenaline was not determined in either group of animals.

DISCUSSION

O'Connor and Verney (10) have shown that painful stimuli in dogs produce two types of inhibition on water diuresis: a rapid, short-lasting inhibition which is pre-

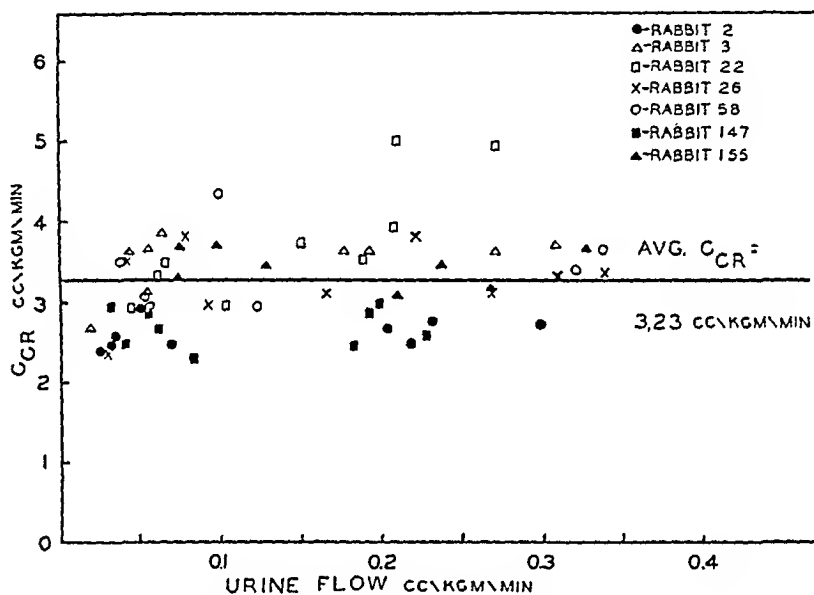


Fig. 3. RELATIONSHIP BETWEEN CREATININE CLEARANCE AND URINE FLOW in undisturbed rabbits. A 16-fold increase in urine flow during water diuresis is possible without significant variation in creatinine clearance. Rabbit 148 (table 3) is not included in this figure because of an unexplained high creatinine clearance averaging 5.98 cc/kg/min.

vented by section of the splanchnic nerves or by denervation of the kidneys and adrenals; and a slow, prolonged inhibition which is abolished by hypophysectomy or section of the supraoptico-hypophysial tracts. Haterius (11) has shown that anti-diuresis is produced in anesthetized rabbits by painful stimulation of the lumbar region and he attributed the phenomenon to increased secretion of the antidiuretic hormone, since the response was abolished by destruction of the pituitary stalk, but he states that, with excessive struggling, antidiuresis may occur in the absence of the pituitary. He also cites a personal communication from Ingrahm to the effect that inhibition of diuresis may occur in cats with diabetes insipidus following unpleasant handling, such as repeated catheterization or gavage. Lippman (12) demonstrated that the painful 'tailcutting' method of obtaining blood in rate reduced PAH clearances by 14 per cent and creatinine clearances by 37 per cent below those values obtained with the painless 'undisturbed' method. Smith (13) and Wolf (14) have

demonstrated reduction in renal plasma flow in man after psychological and physical trauma.

It is clear that antidiuresis may involve either an increased secretion of the antidiuretic hormone or renal ischemia, or both. In contrast to O'Connor and Verney's observations in dogs, our data on rabbits indicate that antidiuresis occasioned by disagreeable stimuli in this species is to be attributed primarily to renal vasomotor changes which cause a marked and prolonged reduction in renal plasma flow and filtration. The absence of any marked increase in the reabsorption of water, as indicated by the creatinine U/P ratio, argues against a significant contribution from the antidiuretic hormone. Apparently marked sympathomimetic activity is easily elicited in the rabbit in contradistinction to the dog.

The locus of vasoconstriction in the rabbit has not been determined, but application of Gomez' (16) equations to our data on renal plasma flow and filtration rate in animals where the blood pressure was measured indicates that both the effective efferent and afferent resistances increase about equally.

Under the conditions of our experiments, one might expect that the renal blood flow might be diverted through the juxtamedullary glomeruli, as described by Trueta and his colleagues (15), in which case one would anticipate a marked reduction in the renal extraction ratio of PAH. Several attempts to measure the extraction ratio by the collection of renal venous blood were defeated by almost complete renal ischemia. However, during oliguria in 4 of our rabbits we obtained good and uniform x-ray visualization of the renal vascular tree following the administration intravenously of a large dose of diodrast. If some of the glomeruli were excluded from the circulation they were uniformly dispersed in areas which still received good circulation through adjacent glomeruli.⁴

Our data show that in a non-excited rabbit a considerable variation in urine flow (0.02 to 0.32 cc/kg/min.) accompanies water diuresis without any related change in filtration rate. Thus the uncomplicated diuretic response appears to be a purely tubular phenomenon, as in the dog and man.

We believe that the parallel variation in urine flow and filtration rate reported by other authors (2-4) is attributable to a reduction in renal blood flow occasioned by the experimental procedures. The majority of observations made by Dicker and Heller (3) and Forster and Maes (4) were made during rising diuresis which corresponds to the recovery phase from the oliguria induced by the administration of water and other procedures necessary to initiate the experiments. Observations made at various stages of this recovery process yield a fortuitous correlation.

SUMMARY

Emotional disturbance and painful stimuli in the rabbit cause antidiuresis by decreasing the renal blood flow (PAH clearance) and filtration rate (creatinine clearance). Increased tubular reabsorption of water (as judged by the creatinine U/P ratio) is of minor importance in this oliguria. The strength of the stimulus needed

⁴ We are indebted to Dr. Charles Gottlieb, Chairman of the Department of Radiology, New York University College of Medicine, for making the facilities of his department available to us.

to produce emotional oliguria varies with the excitability of individual animals, but the mechanism may invariably be provoked by sufficiently strong stimuli. During emotional oliguria, water-intoxication convulsions and death are easily provoked.

When emotional disturbance is avoided, the urine flow may vary 15-fold with a constant filtration rate. Pitressin in physiological doses causes a marked increase in the tubular reabsorption of water with little effect upon renal blood flow. Adrenalin in large doses causes renal ischemia similar to that observed during emotional oliguria. Emotional oliguria may be induced with equal facility in rabbits with enervated and denervated kidneys.

ADDENDUM

Since the completion of this work, Wills and Main (17), working with anesthetized rabbits found constant filtration rates with varying urine flows, thereby agreeing with our results as obtained with unanesthetized and undisturbed rabbits.

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RESPONSE OF RENAL BLOOD FLOW AND CLEARANCE TO GRADED PARTIAL OBSTRUCTION OF THE RENAL VEIN¹

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INVESTIGATION of the rôle of the kidney in the edema of congestive heart failure has suggested a sodium retaining mechanism whereby both glomerular filtration rate and renal blood flow are reduced, the latter more so than the former, while tubular reabsorption of filtered sodium is unimpaired (1, 2). Since glomerular filtration rate is not reduced as much as concurrent renal plasma flow the filtration fraction is elevated. Opinion differs as to whether the increased filtration fraction is due to efferent arteriolar vasoconstriction presumably on a humoral basis (3, 4) or due to high glomerular capillary pressure resulting from the elevated venous pressure which usually accompanies congestive heart failure (5). Merrill dismisses the venous pressure as being unimportant in the renal changes. Bradley and his associates (6, 6a) found, however, that when renal venous pressure in human subjects was elevated from 5.8 mm. Hg to 18.3 mm. Hg. effective renal plasma flow and glomerular filtration rate were reduced on the average by 24.4 and 27.5 per cent respectively. They found no significant change in filtration fraction. Bradley obtained elevation of renal vein pressure by inflation of a pneumatic abdominal girdle to 80 to 100 mm. Hg. This transmitted a pressure of 20 mm. Hg to the abdomen.

Merrill found no significant correlation between venous pressure levels and inulin and hippurate clearances in different patients. Yet in view of the findings of Bradley it would seem likely that alterations in renal vein pressure might have a definite effect on renal clearance when other factors which might influence renal function are kept constant. However, since the abdominal cuff technique might introduce other modifying factors on the kidney function, such as vasomotor influences of psychogenic origin and compression of the ureters and renal pelvis on rate of urine flow, it seemed desirable to investigate the influence of renal vein obstruction on renal function by a technique specific for that factor alone. This was accomplished in dogs by exposure of the renal vein and gradual occlusion by a tourniquet. Renal vein pressure was recorded from a point between the obstruction and the kidney. Effects on either direct blood flow or clearances of creatinine and p-amino-hippurate (PAH) were studied.

METHODS

Dogs were anesthetized with 30 mg/kg. pentobarbital sodium administered intravenously. In one group the influence of venous obstruction on direct renal blood flow was examined with an

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optically recording bubble flow meter by the arterial inflow technique in adequately heparinized animals. This method is described in detail elsewhere (7). In this group, exposure of the left kidney was made by a dorsal retroperitoneal approach. Renal arterial pressure was measured from a point just proximal to the renal arterial cannula. Renal vein pressure was obtained by aid of a specially designed metal sound which reached from the right jugular vein into the left renal vein. Zero levels for venous pressure were set at the level of the inferior vena cava, and for arterial pressure at the level of the renal artery. Pressures were recorded by modified Gregg optical manometers of suitable sensitivity and frequency. To produce elevation in renal vein pressure a tourniquet was passed around the renal vein medial to the end of the inlying renal vein sound. This tourniquet was passed through a rigidly supported brass tube to the exterior to permit gradual uniform constriction of the vein.

In a second group, renal clearances were performed with suitable blood levels of creatinine and PAH maintained by intravenous infusion or subcutaneous injection. Mannitol was included to act as an osmotic diuretic. In these animals a ventral approach to the renal vein was used. The method of measuring renal vein pressure and producing graded venous pressure elevation was the same as above, except that a water manometer was used instead of optical registration for recording pressures. In this group femoral arterial pressure as taken by a mercury manometer was used to calculate the A-V pressure differences across the renal vascular circuit. Urine was collected by direct cannulation of the left ureter. This minimized dead space and emptying errors. The alkaline picrate method (8) was used for creatinine analysis of urine and sodium tungstate plasma filtrates. Analysis for PAH was by the method of Smith (9) on CdSO_4 plasma filtrates and urine. All analyses were made in duplicate. Clearances were calculated by obtaining approximate midpoint plasma values by interpolation to the slope established by samples taken before and after each pair of urine collection periods of ten minutes duration. Appropriate correction was made for emptying delay.

The general plan of the experiment was to raise renal venous pressure in three stages following a suitable control period. In the experiments concerned with direct blood flow, readings were taken every two minutes for a total period of 10 to 12 minutes at each stage, with brief periods intervening for tourniquet adjustment and stabilization of flow. Following the highest level of venous pressure elevation the tourniquet was released and usually two recovery periods were obtained. In the clearance experiments, each phase consisted of two consecutive urine collection periods preceded by adequate discard periods.

RESULTS

Effect of Graded Venous Obstruction on Direct Renal Blood Flow. Five control experiments were performed to establish possible effects of experimental procedures without venous obstruction on blood flow as measured by the bubble flow meter connected between the carotid and renal artery. In all cases blood flow decreased somewhat during periods of time equivalent to experiments in which venous pressure elevation was produced. From this evidence it seemed necessary to compare the renal blood flow during the periods of elevated venous pressure with the expected mean trend, rather than to the initial control value. The basis for this is the fact that the decline in flow observed in the control series is a linear function which can be predicted by a line connecting the initial and final periods. This is illustrated in the representative experiment in figure 1-A. In the five control experiments 25 intermediate periods averaged 0.98 of the predicted trend. The effect of graded venous obstruction was accordingly measured by deviation of flow from the line connecting the control average with the recovery average, and expressing the experimental change as a ratio to this predicted mean trend. In the succeeding discussion this ratio will be referred to as the *experimental/control ratio*, it being understood that the designation 'control' here does not refer to the initial control value, but rather to the adjusted value based on the expected mean trend.

A representative experiment showing the effects on renal flow of graded elevation of renal venous pressure is shown in figure 1B. For three successive stages of venous pressure elevation the experimental/control flow ratio is 0.91, 0.93, and 0.81. Because all seven experiments differed somewhat in control rate of flow (range, 69 to 134 cc/min/kidney; average, 119 cc/min.) and in control renal arterial pressure (range, 72-121 mm. Hg; average, 95 mm. Hg), the changes in flow are combined graphically in figure 2, where percentile deviation from control flow is related to the renal venous pressure. It is seen that effects on flow are significant in 3 experiments

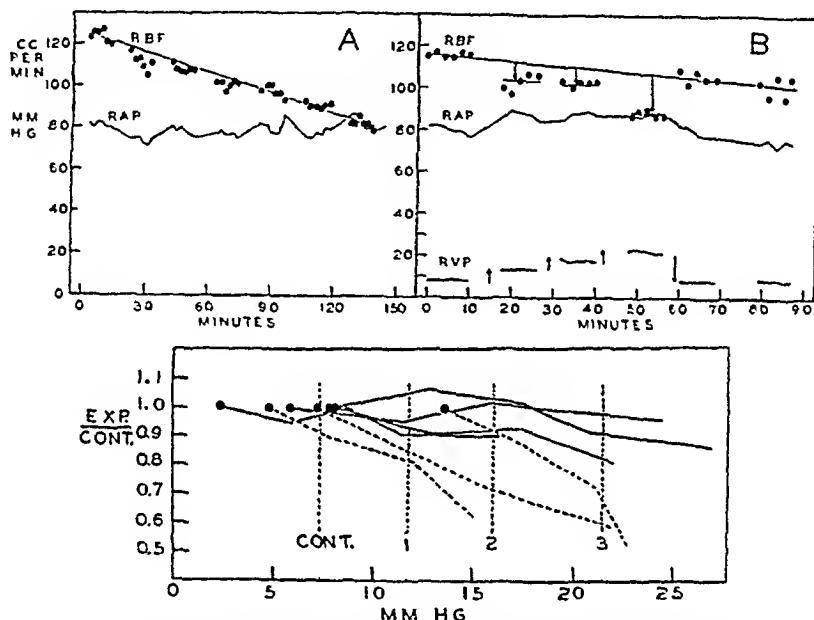


Fig. 1. (*upper*) (A) THE CHANGE IN DIRECT RENAL BLOOD FLOW (RBF) in cc/min/kidney in a typical experimental preparation. This is considered as the 'control' trend, for the gradual decrease in flow noted is due to nonspecific factors other than renal venous pressure elevation. RAP: = renal arterial pressure.

(B) THE EFFECT OF ELEVATION of renal venous pressure (RVP) on direct blood flow. The upper arrows show the degree of reduction of RBF from the expected trend at each stage of RVP elevation.

Fig. 2 (*lower*). SUMMARY OF THE EFFECT OF ELEVATION OF RVP (in mm. Hg on the abscissa) on direct blood flow expressed in a ratio to the control value. Solid circles are the control renal venous pressures. Vertical lines represent the average renal venous pressures of successively the control, first, second, and third stages of venous elevation. The sloping dashed lines show experiments in which it is believed that other factors (neurogenic or humoral) are superimposed on the effect of venous obstruction, accounting for the more marked effect (see text).

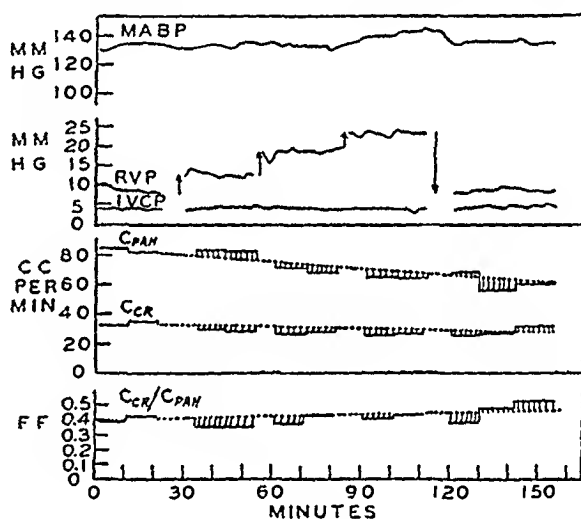
(dashed lines in figure 2) with experimental/control ratios of 0.53, 0.59, and 0.63, but less significant in the other 4 (0.81 to 0.96) at the highest venous pressures. The average decrease in flow for all was 18 per cent in a range of venous pressures from the control average of 7.3 mm. Hg to the average of the third stage of venous elevation, 21.3 mm. Hg. The average decline in pressure gradient across the renal vascular circuit (arterial pressure minus venous pressure) was from 88 to 79 mm. Hg, a 10 per cent decrease.

Calculation of renal vascular resistance from the ratio $\frac{P(A-V)}{F}$ showed increases

of 28, 60, and 80 per cent respectively in the above 3 experiments which showed significant reductions in flow. The other 4 showed no significant changes (± 13 to -10 %), indicating that the decline in flow in these experiments was simply the result of decreased perfusion pressure unaccompanied by change in renal vascular resistance in the range observed. Inasmuch as this is true in 6 experiments to be described subsequently in which renal flow was estimated from the PAH clearance, it can be concluded that the typical effect of elevation of renal venous pressure on the renal circulation is a decline in flow directly proportional to the decrease in effective pressure gradient across the renal circuit. Apparently, humoral or vasomotor factors were superimposed upon the effects of venous obstruction in the above 3 exceptions.

Effect of Graded Venous Obstruction on Renal Clearances. Although study of the renal blood flow by direct methods has advantages in objectivity it was thought that examination of renal clearances under similar experimental conditions would

Fig. 3. EFFECT OF ELEVATION OF RVP ON clearance of PAH and creatinine, and on the filtration fraction (FF). This experiment is one in which the changes in the clearances from the expected trend are minimal. Clearance values are for one kidney. Mean arterial pressure (MABP) was taken at the femoral artery. IVCP: inferior vena cava pressure.



throw light on the rôle of elevated venous pressure on the filtration fraction. This was done to test the postulate that increased venous pressure might increase glomerular capillary pressure and hence the filtration fraction. By using the clearance of PAH to estimate renal plasma flow further information was supplied on the effect of venous pressure elevation on blood flow.

As was done with the data on direct blood flow, it appeared wise to consider the possible effects of experimental procedure other than renal vein obstruction on renal clearances. Data are available from 10 animals observed in connection with another study which were subjected to similar anesthetic and surgical procedures. The average decline in PAH clearance was 15 per cent (range, -1 to -40) and 10 per cent for creatinine (range, 0 to -36 %) during an average time interval of 198 minutes. Intermediate periods (PAH) averaged 93 per cent of the expected trend.

Comparison of the recovery periods following partial venous obstruction with the control averages in the present series showed a decrease of 27 per cent (-6 to -43) for PAH and 20 per cent (± 2 to -39) for creatinine. Although these changes average greater than the above control data there is considerable overlap, and the

view is favored that the changes are due to other factors than the specific effect of renal vein obstruction. For this reason, experimental changes resulting from elevated venous pressure will be compared to the expected trend set by the control and recovery periods, as was done with the direct blood flow experiments.

Two representative experiments illustrating the extremes of results are graphically presented. In figure 3 the trend of PAH and creatinine clearances appears to be only slightly modified by renal venous pressure elevation, while in figure 4 the effect of increased venous pressure is significant. The difference in effects is readily explained on the basis of the change in effective pressure gradient (A-V). In figure 3, due to a fortuitous rise in mean arterial pressure, the net decrease in pressure was only 6.5 per cent during the third stage of venous pressure elevation. This corresponds with a decrease in PAH clearance of only 4.0 per cent. On the other hand, in figure 4 arterial pressure declined during the course of the experiment, and the

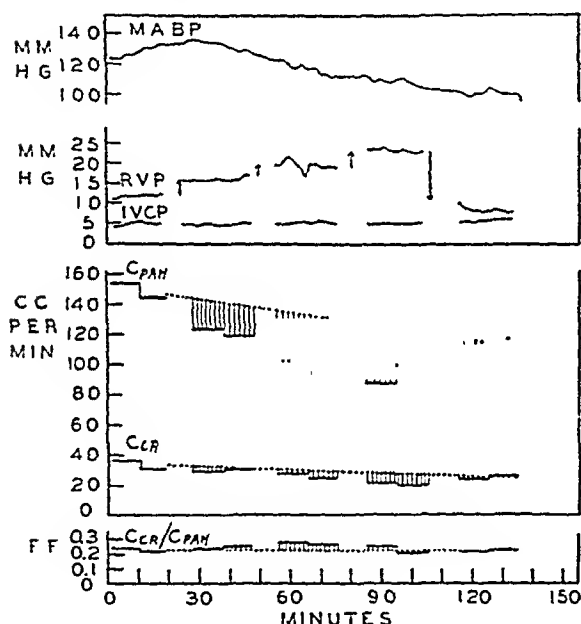


Fig. 4. THIS EXPERIMENT is one in which the effect of elevated RVP on the expected trend of C_{PAH} and C_{CR} was greatest.

A-V pressure gradient decreased by 25 per cent during the third stage of venous elevation; during this phase the PAH clearance was 26 per cent below the expected trend. Thus it is clear that when changes in effective plasma flow occur they are directly related to changes in renal perfusion pressure.

In figure 5 the effects on PAH clearance are graphically combined for all 6 experiments in terms of percentile deviation from the control. As venous pressure was elevated from the control average of 7.7 mm. Hg to 23.5 mm. Hg, the clearance of PAH decreased by an average of 12 per cent from the expected trend: range (-4 to -26 %). The average calculated A-V pressure change across the renal vascular circuit decreased from 125 to 108.5 mm. Hg at the highest venous pressures, a 13 per cent decrease. This signified no change in renal vascular resistance, since flow declined proportionally to the average decrease in effective pressure gradient in all cases.

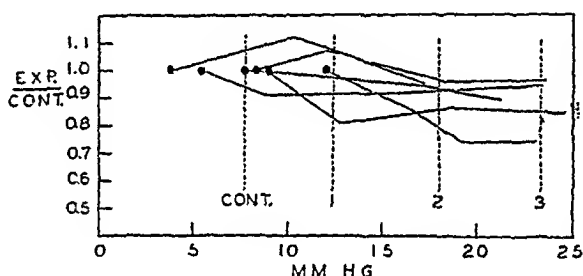
The changes in the clearance of creatinine paralleled the changes in the PAH clearance in individual experiments and the average decrease at the highest venous

pressure was also 12 per cent. Thus it is obvious that there was no significant change in filtration fraction resulting from venous pressure elevation in the range observed.

DISCUSSION

The height of venous pressure elevation in most of these experiments (ca. 29–36 cm. water) was comparable to the degree of venous pressure elevation seen in severe congestive heart failure. Yet with this degree of elevation the reduction in renal blood flow was only 18 per cent in a group of animals in which flow was measured directly, and in another group clearances of PAH and creatinine were reduced only by 12 per cent.² This would appear to dismiss elevated venous pressure as the factor which causes the marked reduction in effective plasma flow (PAH clearance) and glomerular filtration rate in clinical congestive heart failure. It is possible that higher degrees of venous pressure elevation than those investigated would have more significant effects on renal blood flow and glomerular filtration rate, but by extrapolation of the present trend it would appear that venous pressure elevation of at least three times that produced in the present experiments would be required to bring glomer-

Fig. 5. SUMMARY OF THE EFFECTS OF ELEVATED RVP ON the clearance of PAH expressed as a ratio to the control value in successive stages of elevation (vertical lines represent average renal vein pressure values at each stage).



ular filtration down to the 'critical level' at which sodium is retained, about $\frac{1}{2}$ to $\frac{2}{3}$ of normal (10).

Our results agree with those of Bradley and his associates on human subjects in that the reduction in clearances is directly proportional to reduction in perfusion pressure across the renal circuit (P/F ratio remains constant). The finding of the constant filtration fraction is also confirmed. This signifies that there is no significant increase in intraglomerular pressure. The reduction in renal blood flow and clearances has been less than Bradley reports despite a slightly greater absolute elevation in renal vein pressure. However, this may be harmonized by considering the effect of venous pressure elevation in terms of total reduction in pressure gradient across the kidney (i.e., 10 and 13 per cent in the present data as compared with 18 per cent in the data of the human abdominal compression experiments).

SUMMARY

When renal venous pressure is elevated from 7.5 to 22.4 mm. Hg by partial venous obstruction, direct renal blood flow and renal clearances (PAH and creatinine)

²The possibility that blood flow might be maintained during graded renal vein obstruction by opening up of collateral venous channels does not seem very likely in acute experiments. When kidneys were perfused *in situ* in freshly killed dogs via the renal vein (artery occluded) at 30 mm. Hg pressure, the highest flow noted was 0.08 cc/min/gm. of kidney, a negligible figure.

decrease by an average of 15 per cent. This decrease in blood flow and clearances can be explained almost entirely by the decrease in pressure gradient across the renal vascular circuit resulting from increased venous pressure, since arterial pressure remains essentially constant. Thus, the A-V pressure difference decreases by an average of 11.5 per cent.

The clearances of PAH and creatinine show parallel reductions, hence there is no alteration in the filtration fraction attributable to venous obstruction. Therefore, no support can be given to the concept that elevated venous pressure increases intraglomerular pressure, at least not in the range studied. The conclusion follows that the increased filtration fraction noted in congestive heart failure is probably attributable to increased efferent arteriolar resistance rather than to high venous pressure.

The reduction in glomerular filtration rate resulting from experimentally elevated renal venous pressure is not enough to favor sodium retention and, in turn, edema formation. Other mechanisms must be operative in congestive heart failure.

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ENHANCING EFFECTS OF GROWTH HORMONE ON RENAL FUNCTION¹

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WE HAVE previously reported (1, 2) that the considerable falls in diodrast or sodium para-aminohippurate (PAH)² and inulin plasma clearances and in diodrast or PAH Tm resulting from hypophysectomy in dogs are not due to a depression of thyroid, of gonad or of adrenal cortical function brought about by the hypophysectomy; the question as to whether loss of growth hormone or of some further anterior-lobe principle is responsible for the falls was left unsettled. In the present paper it is shown that loss of growth hormone is responsible, since its administration to hypophysectomized dogs raises the depressed values to or above normal and to normal dogs raises normal values to twice the normal.

METHODS

The clearance procedures and chemical methods were as previously described (2). Growth hormone prepared according to the Wilhelmi-Fishman procedure was generously supplied by the Armour Laboratories, Chicago. It was given daily subcutaneously for periods of 9 to 12 days. Except for the last 3 days with K44 we used Armour's lot 3PKR₃, giving 0.5 mg/kg. daily. Armour Laboratories state that 50 gamma/rat/day of this material is sufficient to cause a 20 gm. weight increase in 15 days, that its adrenotrophic and gonadotrophic activities are negligible and that its thyrotrophic potency is estimated to be quite low. Published comparisons of the relative sensitivities of the dog and the rat to growth hormone are few. Putnam, Teel and Benedict (3) found that 1 to 2 cc/kg/day of their extracts produced increased growth in dogs, while increased growth in rats was produced by daily doses of up to 4 cc. per rat. Evans, Meyer and Simpson (4) found that 1 cc. of extract daily to adult female rats regularly stimulated growth of 55 gm. in 20 days. Daily dosage of 25 to 40 cc. of the same type of extract in a hypophysectomized puppy produced growth greater than that of a normal litter-mate control. The hypophysectomized puppy weighed 2.1 kg. at the beginning and 7.3 kg. at the end of the period of injections; it thus received 5 to 12 cc/kg/day as compared with 4 cc/kg/day for the rats. Normal dachshund pups receiving 0.5 to 1 cc. of extract daily grew much faster than litter-mate controls; shepherd pups were less responsive. It thus appears that the effective dose per kg. of dog approximates that per kg. of rat, with considerable variations in different breeds of dogs. Since 0.05 mg. of Armour's lot 3PKR₃ is an effective dose in young rats, this is about 0.5 mg/kg of rat. This dosage was accordingly used in our dogs; it is not at all certain that maximum effects have been attained. For the last three injections on K44 we used Armour's lot 3PKS₃R; the dose was 1.25 mg/kg/day, since it was stated to have approximately one half the potency of lot 3PKR₃.

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² The PAH was generously supplied by Sharp & Dohme, Philadelphia.

RESULTS

Normals. The effects of growth hormone administration on the observed renal functions of 2 normal dogs are shown in table 1. It is seen that PAH and inulin clearances and PAH Tm are greatly increased after 9 or 12 days, although there is little or no effect after 5 days (K43—11/3/48). The values have begun to fall in 2 days and have returned to or slightly below normal by 6 days after cessation of growth-hormone injections.

Hypophysectomized. Table 2 shows that the depressed functions of hypophysectomized dogs are raised to or above normal after 9 days of daily growth-hormone administration; here an effect is apparent after 5 days (K39—11/29/48). The falls in plasma nonprotein nitrogen accompanying the clearance increases should be noted.

TABLE 1. EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN NORMAL DOGS

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH Tm	PLASMA GLU- COSE
<i>Dog K43</i>					
		cc/min/ M ²	cc/min/ M ²	mg/ min/M ²	mg. %
10/28/48	Normal	352	116	20	81
10/29/48	through 11/10/48—0.5 mg/kg. growth hormone (3PKR ₃)				
11/ 3/48	5 days of growth hormone	336	117	27	81
11/10/48	12 days of growth hormone	849	170	33	91
11/12/48		608	138	20	79
11/16/48		308	91	16	80
<i>Dog K44</i>					
12/21/48	Normal	229	78	19	93
12/21/48	through 12/27/48—0.5 mg/kg. growth hormone (3PKR ₃)				
12/28/48	through 12/30/48—1.25 mg/kg. growth hormone (3PKS ₃ R)				
12/30/48	9 days of growth hormone	388	124	35	117

Growth hormone daily subcutaneously.

Adrenalectomized. Table 3 shows that the response to growth hormone of the renal functions of a bilaterally adrenalectomized dog maintained on subcutaneously implanted desoxycorticosterone acetate (DCA)³ pellets is slight or absent. There is a small increase after 5 days of injections (11/1/48) but this is not seen after 12 days (11/8/48). In an effort to discover whether the failure of the striking effects seen in the normal and the hypophysectomized dogs was due here to a deficit of adrenal cortical hormone (failure of DCA pellets to afford adrenal cortical replacement adequate for exhibition of growth-hormone effect) the experiment was repeated with the animal also receiving 0.2 cc. daily of Upjohn lipo-adrenal cortex intramuscularly. Here also no increase was obtained; the result is surprising in that there was an actual decrease of PAH clearance and Tm.

³ The DCA pellets were generously supplied by the Schering Corporation, Bloomfield, N. J.

The failure of this dog to show the enhancing effects of growth hormone seen in normal and hypophysectomized dogs cannot be ascribed to a poor general condition due to adrenocortical deficiency; its appetite, weight, activity and disposition were normal, clearances and PAH Tm were within normal limits, and repeated plasma sodium and potassium values normal; e.g., on 11/24/48 plasma Na and K were 145 and 5.6 mEq/l., on 1/3/49 they were 143 and 5.0. The dog showed, however, the moderately

TABLE 2. EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN HYPOPHYSECTOMIZED DOGS

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH Tm	PLASMA NPN	PLASMA GLU- COSE
<i>K39</i>						
		cc/min/ M ²	cc/min/ M ²	mg/ min/M ²	mg. %	mg. %
6/13/47	Normal	264	95	19		
6/16/47	Normal	263	96	23		
6/20/47	Simple hypophysectomy					
7/17/47		158	63	6.1		
9/10/47		176	44	8.8		
12/31/47		151	45	8.6		66
2/16/48		129	52	5.6	37	71
11/22/48		124	51	6.8		65
11/24/48	through 12/3/48—0.5 mg/kg. growth hormone (3PKR ₃)					
11/29/48	5 days of growth hormone	235	82	17	28	106
12/ 3/48	9 days of growth hormone	261	95	18		99
<i>K42</i>						
11/10/47	Normal	247	77	22		95
12/15/47	Normal	276	95	26		94
1/21/48	Simple hypophysectomy					
2/26/48		130	53	5.1	66	92
3/24/48		124	49	6.5	50	80
12/ 7/48		149	54	9.3		82
12/ 7/48	through 12/16/48—0.5 mg/kg. growth hormone (3PKR ₃)					
12/16/48	9 days of growth hormone	353	110	26	27	121

Growth hormone daily subcutaneously.

elevated plasma nonprotein nitrogen values which we have consistently seen in DCA-supported adrenalectomized dogs even though they showed no other abnormality.

DISCUSSION

The demonstration that growth hormone raises the depressed renal functions of hypophysectomized dogs to or above normal and raises the values of normal dogs to twice the normal makes it unnecessary to postulate some further 'renotrophic' anterior-lobe principle whose loss is mainly responsible for the depression of renal functions resulting from hypophysectomy; our earlier demonstrations that loss of the gonado-

trophic and adrenotrophic hormones is not responsible and that the effect of loss of thyrotrophic hormone is slight (1, 2, 11) restrict the principal effect to loss of growth hormone or of some substance not separated from it. The results are consistent with the finding of an increased urea clearance in acromegaly (5). It may safely be accepted that the growth-promoting principle is formed by the eosinophiles (14); the concept that the principle responsible for the enhancing effects on renal function is formed by the eosinophiles is further supported by the finding that in Cushing's disease, in the absence of renal disease, with basophilic hyalinization which presumably indicates ineffective secretion of the basophile cells there is no depression of renal function (5), and by our earlier findings that dogs with denervated neurohypophysis, resulting in loss of basophiles in the anterior lobe (15), show no persistent depression of renal function (6, 7).

TABLE 3. EFFECTS OF GROWTH HORMONE ON RENAL FUNCTION IN AN ADRENALECTOMIZED DOG

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH TM	PLASMA NPN	PLASMA GLU- COSE
<i>K41</i>						
		<i>cc/min/ M²</i>	<i>cc/min/ M²</i>	<i>mg/ min/M²</i>	<i>mg. %</i>	<i>mg. %</i>
1/21/48	Left adrenalectomy; 3 pellets (75 mg. each) desoxy-					
	corticosterone acetate implanted					
1/28/48	Right adrenalectomy					
2/24/48		213	86	12	37	75
10/25/48		276	96	14	46	85
10/27/48	through 11/8/48—0.5 mg/kg. growth hormone					
	(3PKR3)					
11/ 1/48	5 days of growth hormone	313	110	17		115
11/ 8/48	12 days of growth hormone	265	76	16	48	91
11/12/48	through 11/24/48—0.5 mg/kg. growth hormone					
	(3PKR3) and 0.2 cc. lipo-adrenal cortex					
11/18/48	5 days of hormones	229	89	6.0		74
11/24/48	12 days of hormones	182	96	4.8	57	78

Growth hormone or growth hormone plus lipo-adrenal cortex daily.

The interpretation of the findings of table 3 is not clear. A possible interpretation is that the striking enhancing effects of growth hormone on renal function as seen in the normal and the hypophysectomized dog do not occur in the absence of some adrenal hormone which is not DCA and is not contained in adequate amount in our dosage of Upjohn lipo-adrenal cortex; it would seem more likely to be a cortical than a medullary product. An analogy may be drawn with the need for thyroid substance to obtain renal responses to anterior-lobe administration; Preloban produces considerable increases in normal dogs (11) but only slight and inconsistent increases in thyroidectomized dogs (1). However, the presence of DCA alone affords an adequate background to permit endogenous growth hormone to maintain normal renal function in the adrenalectomized nonhypophysectomized dog (1, 2) and to permit smaller and shorter lasting increases in response to Preloban, as in dog K31 (1), and to growth hormone, as on 11/1/48 of table 3. A further possibility is that smaller doses of

lipo-adrenal cortex would have made possible the full enhancing effects of growth hormone and that the dosage employed was high enough to achieve an effective antagonism between adrenocortical and growth hormones such as has been described for the growth-promoting effect (16). Some support to the view of antagonism may be afforded by the observations that whereas growth hormone raised the fasting plasma glucose levels in the normal and hypophysectomized dogs (tables 1 and 2) and in the adrenalectomized dog supported with DCA alone, it did not when lipo-adrenal cortex was added.

Acute renal hyperemia has been produced by pyrogens, by intravenous amino acid and by intravenous adenine derivatives (8). Chronic renal hyperemia has been produced by high-protein diet in dogs (9) and less strikingly in man (10); it also results from thyroid or anterior lobe administration (1, 11-13). Our present finding of a non-toxic substance which does not raise general metabolic rate or body temperature and which produces striking chronic increases in renal blood flow, filtration rate and tubular activity may be clinically useful in certain conditions where such effects may be desirable.

SUMMARY

Daily growth-hormone administration for 9 to 12 days doubles the PAH clearance and almost doubles the inulin clearance and PAH Tm in normal dogs and raises the greatly depressed values of hypophysectomized dogs to or above the normal levels. It has but slight effect on these functions in the adrenalectomized dog maintained on DCA pellets. In view of our earlier demonstrations that loss of the gonadotrophic and adrenotrophic hormones is not responsible for the great depressions of these renal functions seen after hypophysectomy, and that the effect of loss of thyrotrophic hormone is slight, the principal effect is due to loss of growth hormone or of some substance not yet separated from it. The enhancing effect of growth hormone on renal function may find some therapeutic application.

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COMPARISON OF THE CARBOHYDRATE EFFECTS OF ADRENALIN INFUSED INTO THE FEMORAL VEIN, CAROTID ARTERY, AORTA AND PORTAL VEIN OF RATS¹

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THE effects of adrenalin introduced into a peripheral vein on the distribution of body carbohydrate are well known. The changes produced by adrenalin when infused into other vessels have been poorly studied. This is particularly true of the effects on carbohydrate metabolism, although the circulatory changes have been well observed (1-3). Intra-arterial adrenalin causes circulatory disturbances in the related limb, but general effects are minimal. It is postulated that adrenalin is rapidly destroyed in the tissues, and when introduced into a limb artery, very little passes into the general circulation.

In the present study normal rats were perfused with adrenalin into the femoral vein, the aorta (thoracic and abdominal), and the carotid artery. The substance was also perfused into the portal vein to determine the direct effects on the liver. The changes produced in liver glycogen and blood sugar levels and in muscle glycogen and blood lactic acid were noted.

EXPERIMENTAL

Adult male rats of the Sprague-Dawley strain were used. They were fed in *ad-libitum* diet of Purina Laboratory Chow with Viobin corn germ supplement and were fasted for 48 hours previous to the experiment.

The infusions were made through a no. 26 hypodermic needle cemented into a No. 4 French ureteric catheter. This was attached to a 2-ml. syringe, the plunger of which was driven at a constant rate by an electric pump (manufactured by the Johnson Foundation, University of Pennsylvania).

Adrenalin hydrochloride solution (1:1,000 Parke Davis) was diluted 1:250 or 1:500 with 0.9 per cent sodium chloride solution, and glutathione (2 mg/10 ml. infusion fluid) was added. Heparin (one drop to 10 ml. solution) was included for the intra-arterial infusions. The solution was infused for 60 minutes, a total volume of 1.6 to 1.8 ml. being given. The dosage was approximately 3×10^{-5} mg/100-gm. rat/minute.

The rats were anesthetized with intraperitoneal Nembutal solution in a dose of 5 mg/100-gm. rat. Blood samples of 0.2 ml. were taken from the tail, and the infusion was then started and allowed to run for one hour. A further blood sample

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was then taken, and the gastrocnemius muscle removed for analysis. The abdomen was then opened and the left lobe of the liver removed for glycogen analysis. Body temperature was maintained during the observation by an electric bulb placed over, but not too close to the rat. The experiments were usually done in pairs on the same day; in one animal adrenalin was infused while in the other control observation 0.9 per cent sodium chloride solution was infused for one hour.

Femoral Vein Infusions. These infusions were made directly into the exposed vein.

Carotid Artery Infusions. With the head extended, a midline cervical incision was made through skin and fasciae and the left sternomastoid muscle retracted laterally. The carotid sheath of vessels was exposed and more fully visualized by incision of the omohyoid muscle as it crosses these vessels. The carotid artery being isolated, a cotton suture was threaded under it and used as a retractor. The needle was inserted in a cephalic direction, and the infusion commenced. The wound was covered with a gauze swab moistened with warm normal saline.

Aortic Infusions: Abdominal Aorta. A lower midline abdominal incision was made. The intestines were displaced to the right, and the aorta exposed by a small incision through the peritoneum of the posterior abdominal wall. A point was chosen in the aorta below the renal artery, and the needle rapidly inserted in a cephalic direction. In a few instances this procedure resulted in some hemorrhage; these experiments were discarded. The infusion was started and the skin and muscles of the abdominal wall brought together by a skin clip. The wound was covered with a gauze swab moistened with saline.

Aortic Arch. The abdominal aorta was exposed as described above. A small 'bull dog'-clamp was applied to the aorta just below the renal artery. The aorta was now ligatured just above its point of division into the common iliac arteries. A further loop-ligature was passed around the aorta. A slit was made in the aorta just proximal to its ligature, and a fine Vinylite tube inserted. The 'bull dog' clamp was removed and the tube pushed along the aorta for a distance of approximately 9 cm. At this distance a slight resistance was encountered, and subsequent dissection showed that the tip of the tube rested in the arch of the thoracic aorta. The tube is kept in position by tightening the loop ligature. If the manipulations are rapid, there is surprisingly little bleeding. The tubing is attached to the syringe, and infusion of adrenalin or of saline is started. It is thus possible to infuse the solution, against the direction of blood flow, directly into the aortic arch.

The blood supply to the lower limbs is greatly altered by ligature of the aorta. Due to the anoxic conditions in the lower limb, analysis of the tail-blood sample showed a conspicuous rise in lactic acid and fall in sugar levels. In these experiments, therefore, the final blood sample was taken from the heart.

Blood glucose was determined by the Nelson (4) method and blood lactic acid by the method of Barker and Summerson (5). Hepatic and muscle glycogen values were estimated by a modification of the Good, Kramer and Somogyi procedure (6).

RESULTS

Femoral Vein Infusion. Compared with the control group, adrenalin infusions resulted in a conspicuous rise in blood glucose and lactic acid levels. Hepatic glyco-

gen increased, and muscle glycogen diminished. Doubling the concentration of adrenalin resulted in an approximately two-fold rise in blood glucose, but the blood lactic acid level showed no significant difference from the smaller dose. The muscle glycogen values were, however, lower. The rise in liver glycogen is presumably related to the deposition of lactic acid released from muscle.

Carotid Artery Infusions. The control group in this series showed greater changes than controls given saline intravenously. The operative procedure had, therefore, a greater general effect than infusion of saline into the femoral vein. The infusion of adrenalin resulted in a slight increase in blood glucose and lactic acid concentrations. Muscle and liver glycogen values showed no conspicuous change. The findings do not support the postulate that the effects of adrenalin on carbohydrate metabolism are primarily mediated through any structure in the vascular territory of the carotid artery.

Portal Vein Infusions. The control observations show that saline infused into the portal vein results in a slight increase in blood glucose levels. Muscle glycogen and blood lactic acid values are not altered. When adrenalin is infused, there is a slight but insignificant rise in blood sugar. Hepatic and muscle glycogen and blood lactic acid are not significantly altered. However, as the liver glycogen levels in both the test and control groups were rather low, the experiments were repeated on animals previously fed glucose (table 1). This resulted in higher liver glycogen values. The results obtained were essentially similar to those found when the hepatic glycogen levels were low.

Aortic Infusions: Abdominal Aorta. Control observations show that the operation alone with infusion of saline produced little change in the distribution of carbohydrate. The infusion of adrenalin into the abdominal aorta resulted in only slight glycemia. The change in hepatic glycogen was not significant. However, there was a conspicuous rise in blood lactic acid values and a fall in the level of muscle glycogen. The adrenalin had therefore resulted in glycogenolysis in muscle. Almost identical results were obtained when the dose of adrenalin was halved.

Aortic Arch Infusions. Although adrenalin infused into the portal vein produced little effect on liver glycogen, it seemed possible that infusion into the hepatic artery might be effective. This procedure would offer great technical difficulties in the rat. However, infusion into the thoracic aorta would indirectly perfuse the hepatic artery through the coeliac axis. When this was done, the changes in blood glucose were not so great as perfusion into the femoral vein. The hepatic glycogen values were not significantly different from those of control values. Ligation of the abdominal aorta necessarily diminishes the vascular bed of the body. When this is taken into consideration, it seems that there is little difference between the changes in liver glycogen and blood glucose values produced by infusion of adrenalin into abdominal or thoracic aorta or into the portal vein. Aortic arch infusion, however, produces very complex changes in the distribution of body carbohydrate. The anoxic state of the lower limbs has already been mentioned; the low muscle glycogen values in both test and control values reflects this anoxia. However, the obstruction produced by the Vinylite tubing to blood flowing down the aorta was probably not very great. Histological sections of liver taken from control group animals at the conclusion of the experiment

TABLE 1. EFFECT OF ADRENALIN INFUSED INTO FEMORAL VEIN, CAROTID ARTERY, ABDOMINAL OR THORACIC AORTA, AND PORTAL VEIN ON BLOOD GLUCOSE, BLOOD LACTIC ACID AND LIVER AND MUSCLE GLYCOGEN LEVELS

SITE OF INFUSION	SOLUTION INFUSED	CONCENTRATION	NO. RATS	BLOOD GLUCOSE MG/100 ML.		BLOOD LACTIC ACID MG/100 ML.		GLYCOGEN %	
				Control Change		Control Change		Liver	Muscle
Femoral vein	Saline		6	76	1.1 ± 2.6 ¹	23.6	-7.2 ± 1.1	387 ± 22.4	504 ± 26.9
	Adrenalin	1:500,000	6	76	37.0 ± 2.8	26.8	8.3 ± 2.6	870 ± 65.2	415 ± 14.2
	Adrenalin	1:250,000	7	72	74.3 ± 8.8	23.9	10.3 ± 3.4	477 ± 28.1	318 ± 20.7
Carotid artery	Saline		6	71	7.3 ± 3.3	19.3	-3.1 ± 1.7	489 ± 48.2	508 ± 17.2
	Adrenalin	1:250,000	7	71	30.0 ± 2.9	21.2	3.1 ± 0.8	492 ± 31.9	449 ± 16.7
Abdominal aorta	Saline		5	70	3.6 ± 3.1	21.9	-7.0 ± 0.8	442 ± 51.8	469 ± 19.8
	Adrenalin	1:500,000	6	73	16.0 ± 4.2	26.6	13.7 ± 5.4	466 ± 42.1	313 ± 22.3
	Adrenalin	1:250,000	6	74	17.0 ± 5.3	23.6	10.0 ± 2.2	322 ± 11.3	326 ± 23.2
Arch of aorta	Saline		5	65	3.8 ± 1.0	21.9	-12.6 ± 2.2	348 ± 22.8	253 ± 39.1
	Adrenalin	1:250,000	6	66	37.1 ± 4.7	19.9	-6.0 ± 0.7	274 ± 26.5	269 ± 23.8
Portal vein	Saline		5	64	5.8 ± 2.1	20.9	-8.2 ± 0.6	188 ± 24.2	515 ± 20.4
	Adrenalin	1:250,000	6	66	17.3 ± 2.8	21.9	-8.1 ± 1.1	218 ± 19.8	485 ± 18.7
	Saline		5	83	7.6 ± 2.2	21.7	-4.5 ± 0.7	1613 ± 43.8	605 ± 44.5
	Adrenalin	1:250,000	5	88	13.0 ± 3.5	24.8	-7.2 ± 1.0	1598 ± 52.8	572 ± 40.5

¹ Mean ± standard error of mean. ² Animals given 5 ml. of 50% glucose solution by stomach tube 18 hours before the experiment.

showed no significant pathological changes. Any profound disturbance of the hepatic circulation, therefore, seems unlikely.

DISCUSSION

The infusion of adrenalin into a peripheral vein produced changes in carbohydrate distribution compatible with those of other workers using similar dosage (7). However, when adrenalin was introduced into an artery or into the portal vein, quite different changes were produced. The substance was now delivered directly to the organ concerned. Adrenalin is destroyed by tissues very rapidly and remains for only a short time in the blood stream. The liver probably has the greatest power of inactivation (8, 9). Intraportal or intra-arterial adrenalin would be expected to cause changes in the territory of the vessel infused. The substance would then be destroyed in the tissues, and if the dose was not excessive, general effects would be minimal.

In the case of the abdominal aorta, the above sequence of events apparently occurred. A direct action on the lower limb muscles resulted in glycogenolysis and consequent increase in blood lactic acid concentration. The adrenalin is infused only into the lower limb muscles, and the changes recorded in muscle are therefore more conspicuous than when the same dose is given intravenously. The slight rise in blood sugar is attributed to failure of tissue destruction of all the adrenalin, some passing into the general circulation. These direct effects of adrenalin on skeletal muscle in the rat are contrary to those described for man. The infusion of adrenalin into the femoral artery of man produces no change in the glycogen content of the ipsilateral gastrocnemius muscle, and the lactic acid content of femoral vein blood from the same limb is not increased (10). This discrepancy is not explainable on the basis of dosage. The dose used in man, after one hour's intravenous infusion, produced a rise in blood sugar of 52 mg/100 ml. (11). This compares well with the blood sugar changes recorded with intravenous infusion in the rat. Borysiewicz (12) injected adrenalin into the femoral artery of dogs and found a normal glycemic response but no change in blood pressure. Baudouin and coworkers (13) also in dogs, using the infusion technique, report that twice to four times the intrafemoral arterial dose of adrenalin was needed to produce the same glycemia as when given intravenously. In neither of these papers were the blood lactic acid and muscle glycogen values recorded.

The failure of adrenalin introduced into the portal vein to discharge liver glycogen, even when hepatic glycogen stores were adequate, was most unexpected. As adrenalin is readily destroyed by the liver, there was little effect on muscle glycogen. In dogs Borysiewicz (12) reported that the blood sugar rise is identical, whether the adrenaline is given into a peripheral or mesenteric vein, whereas Baudouin *et al.* (13) showed that twice the dose of intramesenteric venous adrenalin was needed to produce the same rise of blood sugar as when given into a peripheral vein. Perfusion of the isolated dog's liver with adrenalin is known to cause glycogenolysis (14, 15). None of these observations is readily comparable with the present work, the species used, the type of anesthesia, the techniques of administration, and the dosage of

adrenalin being very different. Circulating adrenalin normally reaches the liver through the hepatic artery. However, mixing with blood occurs in the hepatic sinusoids, and it is with sinusoidal blood that the individual liver cells come in contact. However, infusion of adrenalin into the aortic arch so that the liver cells received the substance *per via naturalis* also failed to cause glycogenolysis. It seems that in the rat adrenalin does not exert a direct effect on liver glycogen. It might be postulated that the liver is affected secondarily to the changes in muscle. The failure of intra-aortic infusions to discharge liver glycogen even when the glycogenolysis in muscle is vigorous makes this unlikely.

Adrenalin is believed to activate the anterior pituitary to release adrenocorticotrophic hormone (16). As adrenalin infused into the portal vein failed to directly affect liver glycogen, it seemed possible that some effects might be mediated through the central nervous system or pituitary and adrenal cortex. However, infusion of the carotid artery with presumably delivery of a large amount of adrenalin directly to the brain failed to produce the effects obtained by infusion into the femoral vein. Moreover, the glycemic response to intravenous adrenalin is normal in hypophysectomized rats (7). The diminished metabolic response to adrenalin when the substance is infused into the carotid artery is probably due to its destruction in the tissues of the head and neck, very little passing into the venous side to affect liver and muscle glycogen.

SUMMARY

Adrenalin infused into the femoral vein of adult male rats causes a diminution in muscle glycogen, an increase in blood lactic acid and blood glucose levels, and an increase in hepatic glycogen concentration. Adrenalin infused into the abdominal aorta caused a depletion of the glycogen of the gastrocnemius muscle with a rise in blood lactic acid, but little change in blood sugar or hepatic glycogen. Adrenalin infused into the arch of the aorta resulted in only slight glycemia with little effect on liver glycogen. Adrenalin similarly infused into the portal vein was without effect on hepatic glycogen, lactic acid, or muscle glycogen. The increase in blood sugar was small. Intra-carotid artery infusions of adrenalin resulted in only slight hyperglycemia and lactic acidemia with no significant change in muscle or liver glycogen.

Adrenalin introduced into an artery is believed to be rapidly destroyed by the tissues into which it is delivered. It exerts a direct glycogenolytic effect on muscle but in the rat apparently has no direct effect on liver glycogen.

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EFFECT OF HORMONES OF THE POSTERIOR PITUITARY ON TOLERANCE OF THE EVISCERATED RAT FOR GLUCOSE

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STUDIES from this laboratory (1) have shown that epinephrine can affect the glucose tolerance of the eviscerated rat and that the nature of the response is related to the experimental conditions. When glucose without insulin was administered to eviscerated rats, the addition of epinephrine did not affect glucose tolerance during the first 2 hours but during 24 hours there was an increase in glucose requirement. When glucose with insulin was administered to eviscerated rats, the addition of epinephrine caused a decrease in glucose tolerance within 2 hours which continued throughout 24 hours. In the present studies it was found that an extract of posterior pituitary affected glucose tolerance in the same manner as did epinephrine. A more highly purified preparation of the pressor principle of the posterior pituitary had similar effects and a preparation of the oxytocic principle which was not free from pressor activity had a relatively weak effect upon glucose tolerance when studied during a period of 2 hours.

METHODS

Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 (± 2) grams they were anesthetized (intraperitoneal injection of 18 mg. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (2).

Solutions containing 0.9 per cent sodium chloride and varying concentrations of glucose (C.P. Dextrose, Merck) with and without regular insulin (Lilly) (4 U/24 hr./rat) were infused into the saphenous vein of the right hind leg by means of a constant injection machine which delivered fluid from each of 6 syringes at the rate of 20 cc. in 24 hours. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mg/100/hr.). The infusions covered periods of 2 and 24 hours. Analyses of glucose by the method of Miller and Van Slyke (3) were made on jugular vein blood taken at the end of the infusion periods.

The extracts of posterior pituitary were added to solutions of glucose and insulin which were given intravenously. The preparations tested were posterior pituitary extract (Upjohn) 20 I.U. per cc.; pressor principle (Pitressin, Parke, Davis) 20 pressor units per cc.; and oxytocic principle (Pitocin, Parke, Davis) 10 I.U. per cc.

EXPERIMENTS AND RESULTS

Experiment 1 (fig. 1) was a study of the effect of posterior pituitary extract (Upjohn) upon the glucose tolerance of the eviscerated rat. Twelve pairs of rats were represented in each group.

Three groups of rats were given a glucose load of 64/100/hr. with insulin for a period of 2 hours. Dilutions of posterior pituitary extract of 1, 2 and 4 parts per 100 each caused a significant rise in the level of blood glucose as compared to that of the control animals. Two groups of rats were given a glucose load of 16/100/hr. without insulin for a period of 2 hours. Dilutions of posterior extract of 1 and 2 parts per 100 had no significant effect upon the level of blood glucose. Two groups of rats were given a glucose load of 40/100/hr. with insulin for a period of 24 hours. Dilutions of posterior pituitary extract of 1 and 2 parts per 100 each caused a significant rise in the level of blood glucose as compared to that of the control animals.

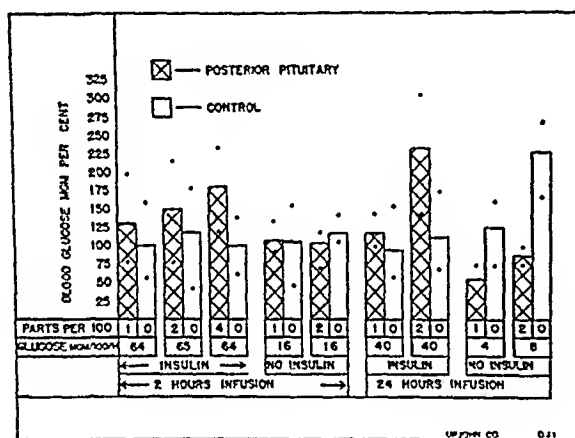


Fig. 1. EFFECT OF POSTERIOR PITUITARY EXTRACT upon the level of blood glucose at the end of the infusion period. Averages and range. Twelve rats per group.

One group of rats was given a glucose load of 4/100/hr. without insulin for 24 hours. A dilution of posterior pituitary extract of 1 part per 100 caused a significant decrease in the level of blood glucose as compared to that of the control animals. A second group of rats was given a glucose load of 8/100/hr. without insulin for 24 hours. A dilution of posterior pituitary extract of 2 parts per 100 caused a significant suppression of the blood glucose level as compared to that of the control animals.

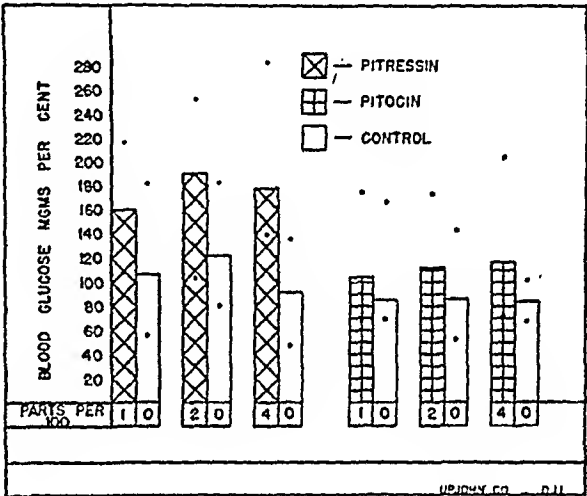
Experiment 2 (fig. 2) was a study of the effect of the pressor and oxytocic principles upon the glucose tolerance of the eviscerated rat. Twelve pairs of rats were represented in each group.

Three groups of rats were given a glucose load of 64/100/hr. with insulin for 2 hours. Dilutions of pressor principle of 1, 2 and 4 parts per 100 each caused a significant rise in the level of blood glucose as compared to that of the control animals. Three groups of rats were given a glucose load of 64/100/hr. with insulin for 2 hours. Dilutions of the oxytocic principle of 1, 2 and 4 parts per 100 each caused a small but definite rise in the level of blood glucose as compared to that of the control animals. After demonstrating that the preparation of the oxytocic principle had an effect upon glucose tolerance similar to that of the pressor principle, this preparation was assayed for its effect upon the blood pressure of the cat. According to this criterion the prep-

aration of oxytocic principle used by us contained approximately 20 per cent as much pressor principle per unit volume as did the preparation of the pressor principle.

Each of the 3 preparations of posterior pituitary hormones used in this study contained 0.5 per cent of chlorobutanol as a preservative. Twenty-four pairs of eviscerated rats were given 64/100/hr. of glucose with insulin for 2 hours. The infusion fluid for one rat of each pair contained chlorobutanol in a concentration of 0.02 per cent which equaled the highest concentration in the dilutions of posterior pituitary preparations. The average blood glucose value for the rats receiving chlorobutanol was 104 mg. per cent as compared to the average of 111 mg. per cent for the control animals. It was therefore concluded that chlorobutanol was not the cause of

Fig. 2. EFFECT OF THE PRESSOR and oxytocic fractions of posterior pituitary extract upon the level of blood glucose at the end of two hours of infusion. Averages and range. Twelve rats per group. Glucose load of 64/100/hr. with insulin.



the rise in blood glucose when posterior pituitary preparations were administered under identical conditions.

DISCUSSION

These studies show that posterior pituitary extracts affect the glucose tolerance of the eviscerated rat in the same manner as does epinephrine (1). When glucose without insulin was administered to eviscerated rats for a period of 2 hours, the addition of posterior pituitary extract did not have any significant effect upon the level of blood glucose but during a period of 24 hours the addition of posterior pituitary extract caused a significant suppression of the level of blood glucose. When glucose with insulin was administered to eviscerated rats, the addition of posterior pituitary extract caused a significant rise in the level of blood glucose during periods of 2 and 24 hours.

The preparations of pressor and oxytocic principles were tested only in eviscerated rats given a glucose load of 64/100/hr. with insulin for a period of 2 hours. The preparation (Pitressin) which was high in pressor activity caused a marked rise in blood glucose just as did epinephrine (1) and posterior pituitary extract (*exp. 1*). The preparation of oxytocic principle (Pitocin) had a less marked effect which roughly paralleled the extent of its contamination with the pressor principle.

The mechanism whereby these principles which act upon smooth muscle also

affect glucose tolerance is not known to us. The problem is made more complex by the fact that direction of the response is reversed by the presence or absence of insulin. Also, it is not known whether the effects of these hormones upon glucose tolerance in the eviscerated rat represent physiological mechanisms of action or whether they should be considered as pharmacologic responses.

SUMMARY

Eviscerated rats were infused intravenously with glucose with and without insulin for periods of 2 and 24 hours. When glucose without insulin was given, the addition of posterior pituitary extract did not have a significant effect upon glucose tolerance within 2 hours but during a 24-hour period there was a significant suppression of the level of blood glucose. When glucose with insulin was given, the addition of posterior pituitary extract caused a significant rise in the level of blood glucose during periods of 2 and 24 hours.

Preparations of the pressor and oxytocic principles with insulin were tested in eviscerated rats during a period of 2 hours. The preparation of the pressor principle caused a marked rise in blood glucose. The preparation of the oxytocic principle had a less marked effect which roughly parallel the extent of its contamination with the pressor principle.

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EFFECT OF METABOLIC INHIBITORS ON MEMBRANE POTENTIALS IN THE SYNOVIALIS OF THE DOG¹

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A PREVIOUS report (1) on the electrochemical properties of synovial tissues was concerned with the diffusion potentials observed with many ions relatively inactive with respect to tissue metabolism. The evidence for their inactivity included the agreement within reasonable limits of the observed membrane potentials with those estimated from the aqueous mobilities of the ions. The calculations were based on the theories of Planck (2) and of Henderson (3) on the nature of potentials at diffusion boundaries. In agreement with theory were the potentials of the alkali and alkaline earth chlorides, the potentials of NaCl in relation to its concentration, and the potentials of bromide, sulphate, salicylate and benzoate ions. Among the halogens, only iodide ion was found to deviate greatly from the potential estimated from its aqueous mobility. These results, with those on thiocyanate and some salicylate derivatives, appear to indicate, at least in part, metabolic reactivity rather than diffusion effects.

The investigation has been continued with the purpose of revealing the effect on the membrane potential of substances which have well-defined effects on metabolism such as inhibitors or activators of various types. By altering in a known manner the pathways of oxidation-reduction processes through known enzyme systems, such as the Warburg-Keilin system (cytochrome-cytochrome oxidase), the relation of the processes to the observed potentials may be determined. Conversely, unknown or hypothetical metabolic processes may be found, and their relative importance established by reference to the membrane potential as a criterion. It is our purpose to determine the nature of the relation of the membrane potential to metabolism.

EXPERIMENTAL

The method of determining the synovial membrane potential in the knee joint of dogs has been described (1). A slight modification has been introduced to permit duplicate determinations on both knee joints with one pair of saturated KCl-calomel electrodes, rather than the two pairs of Ag-AgCl electrodes previously employed. This procedure eliminates the need of correcting small errors due to differences in the reference electrodes. The indicator electrode was connected to approximately isotonic (0.15 M) solutions within the joint cavity by means of the technique previously described. By using an extension bridge of saturated KCl, this electrode

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¹ The expenses of this investigation were borne by the Arthritis Research Project of the University of Illinois. A preliminary report was presented before the American Physiological Society, Minneapolis, September 17, 1948.

was connected to the solution being observed in either joint. The subcutaneous needle was filled with NaCl solution (0.15 M), and connected to a syringe barrel containing the same solution. These were connected to the reference calomel electrode by means of a saturated KCl bridge. In most experiments the procedure was to vary the solutions within one joint cavity, making EMF readings with every solution with time. The other joint was used as a control with NaCl (0.15 M). Later the observations were repeated on the control joint.

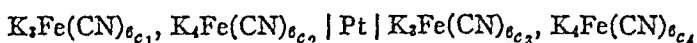
With a given solution, the fluid was aspirated and replaced a sufficient number of times to yield fairly stable, reproducible potentials. With numerous reagents, drifting potentials were obtained, but these usually reached a fairly steady state, often passing through a maximum or minimum value near which the potentials were stable. The behavior of these potentials was roughly characteristic of each inhibitor or activator. The most reproducible features were the levels at which the initial potentials appeared, the levels at maxima or minima, and the final stable levels which developed after long exposure. Differences of these levels between various inhibitors were generally of a larger order of magnitude than the range of potentials over which any drift occurred.

All inhibitor or activator solutions were made up to isotonic strength with NaCl. The sodium salts of the following substances were included: cyanide, sulphide, azide, malonate, o-iodobenzoate, iodoacetate, thioglycolate, fumarate, succinate, iodide, and thiocyanate. Cations included ferric, cupric, mercuric and hydroxylamine, introduced as the chlorides. Neutral substances included thiourea, urea, thioracil, hydrogen peroxide and molecular iodine. The effects of methylene blue also have been observed.

The experimental results are presented in tables 1 to 5, in which the reagents are classified according to the following types: 1) inhibitors of cytochromeoxidase; 2) thiols; 3) heavy metals and other sulfhydryl inhibitors; 4) inhibitors and activators of succinic dehydrogenase; and 5) electron acceptors from cytochromeoxidase. Of course, most active substances fall into more than one group. Regardless of this fact, the classification is convenient and a useful first approximation, but it represents what appears to be the most predominant effect of the substance rather than the totality of its effects. The potentials which are given represent the averages selected according to some characteristic indicated in the tables. These are usually the initial potentials, or the most stable final potentials, depending on which characteristic is the more reproducible.

Theoretical Considerations. The membrane potentials which have been observed fall into two distinct classes. Those produced by the alkali and alkaline earth cations, by chloride, bromide, sulphate and other anions, are of the order of plus or minus 10 millivolts or less, compared with NaCl at isotonic concentrations. They are of the sign and magnitude predicted by the Planck-Henderson theory of diffusion potentials taking accepted values of the relative ionic mobilities and Hittorf transference numbers, as determined from conductance and transference data in aqueous solution (1). The potentials produced by cyanide, sulphide, ferric, cupric and other ions, known to act as metabolic inhibitors or activators, are of a much larger order of magnitude, greater than 400 millivolts in some cases, even at low concentrations compared with

the isotonic NaCl present in the solutions. The theory of diffusion potentials does not account for potentials of that order of magnitude under the conditions of the experiments. These potentials are to be classed as metabolic potentials, as distinguished from the diffusion potentials observed with metabolically inactive ions. In every case both factors, ionic mobility and metabolic effect, contribute to the observed potential, but the metabolic effect, when present, results in a potential that may be of a greater order of magnitude. In order to relate the experimental results to known effects of the inhibitors and activators, the following considerations are presented. Boundaries between two oxidation-reduction systems may be imagined to approach either of two limiting conditions: 1) boundaries at which occur oxidoreductions involving electron exchange but not ionic transference; and 2) those at which ionic transference takes place, but not electron exchange. The first will be termed 'boundaries without transference'. This type is exemplified by the model



in which platinum is the only connection between the solutions. When the potential difference is measured with a pair of identical KCl calomel electrodes connected to the two solutions, the electrochemical process is the reduction of ferricyanide and oxidation of ferrocyanide in the solution of lower redox potential. In the other solution the reaction goes in the opposite direction. The electromotive force, E , is given by the relation

$$E = \frac{RT}{F} \ln \frac{a_1 a_1}{a_2 a_3} \quad (1)$$

R denotes the gas constant, T the absolute temperature, and F the Faraday electrochemical equivalent, 96,500 coulombs; while a denotes the thermodynamic activity of an ion at the concentration c . In other words, the boundary potential can be calculated as the difference between the redox potentials of the two solutions measured individually. Thus

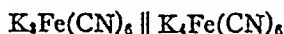
$$E = E_t - E_s = E_s \quad (2)$$

where E_s and E_t are the potentials at the electron source and terminal, respectively. The former represents the redox potential of the reducing solution and the terminal potential refers to that of the oxidizing solution.

The mechanism by which electrochemical oxidoreductions in tissues occur with electron exchange at boundaries will be assumed to be that of aerobic respiration over the cytochromes. Within recent years it has been generally accepted that this process involves the alternate oxidation and reduction of the individual ferro- and ferri-porphyrin enzymes, whereby electrons originating in the substrate first reduce one of the acceptor components, eventually reducing ferri-cytochrome oxidase, the respiratory enzyme of Warburg, and being accepted by molecular oxygen (4). Unlike many other enzymes, the cytochromes with the exception of the component c are quite insoluble. All occur predominantly as part of the insoluble structures of cells and tissues. The process of aerobic respiration over the system can be regarded, accordingly, as a mechanism involving the conduction of electrons from substrate to

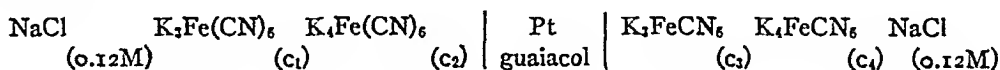
oxygen rather than as one in which the iron enzymes migrate. Szent-Györgyi (5) has described this as a series of quantum jumps, involving definite energy levels in the cytochrome system, and producing corresponding quanta of radiant energy. A somewhat related conception of electron conduction in other iron porphyrins has been developed (6). With the cytochrome system as conductor, a biological phase boundary at which tissue respiration occurs, can be represented as a boundary without transference analogous to the model. The electron source is the substrate, yielding electrons to cytochrome *b*, *c* or *a* in order of increasing standard potentials. The terminal is the oxygen-ferro-ferricytochrome oxidase system. The source, cytochrome *b*, for example, is represented in the model by ferrocyanide, and the terminal, cytochrome oxidase, by ferricyanide. The platinum conductor corresponds to the electron conducting system, represented in the tissues by the cytochromes acting as a whole.

As a next approximation the other limiting case, that of ionic transference, is considered. A 'boundary with transference' is exemplified by the model



where the double vertical lines represent an unspecified type of boundary between the oxidized and reduced ions. Processes involving electron exchange independent of ionic diffusion are excluded. The potential at such a boundary may be one of several types: diffusion (2,3); oil phase (7), or membrane with selective permeability (8). In any case the potential is determined primarily by the mobilities or transference numbers of the various ions without regard to the redox potentials. The electrical resistance at such a boundary is low in the case of a diffusion boundary, high in the case of an oil-phase boundary.

An electrochemical model applicable to tissues should include the properties of both models, with and without transference. Such a boundary will be termed a 'boundary with partial transference.' In particular it will be considered to have a high resistance. Earlier measurements have indicated the resistance of the synovial membrane to be of the order of 100,000 ohms (1). To illustrate some of the properties of the boundary with partial transference, the potentials of the following system were determined:



The two solutions were separated by a layer of guaiaicol of variable thickness. They were connected by a platinum wire, serving as electron conductor. The potential was measured by means of identical saturated KCl calomel electrodes making liquid junctions with the two solutions. The ratio of concentrations c_1 to c_2 was 10.0 and that of c_3 to c_4 was 0.1. The total concentration of ferro-plus ferricyanide was 0.02 M in each solution. The theoretical EMF of the boundary without transference at 25°C. is

$$\begin{aligned} E &= 0.05915 \log \frac{c_1 c_4}{c_2 c_3} \\ &= 118.3 \text{ millivolts} \end{aligned}$$

where concentrations are used instead of activities. This is approximately correct, because the ionic strengths of the two solutions are very nearly the same.

Measurements of this system gave 116 mv. for a thick guaiacol layer. As the thickness of the layer was continuously decreased, the potential difference fell continuously to a value of approximately 50 mv. for a thin film of guaiacol, at which point the potential broke and approached zero. For a cellophane membrane of much lower resistance, the potential difference was 3 mv. Thus the potential difference of a boundary with partial transference can be made to approach that of a boundary without transference when the conduction is made vanishingly small. For this case *equation 2* determines the boundary potential.

In applying these principles to the experimental results, the following definitions are introduced. The mean source potential, \bar{E}_s , is defined as the mean potential at which electrons are accepted by the cytochromes from substrate via enzymes and carriers. At a given instant it is the potential averaged over all electrons, each of which is assumed to enter the cytochrome system at a potential which varies from point to point and from instant to instant. The mean is taken over all acceptor cytochromes, normally b, c, and a. \bar{E}_s is related to E_s , the source potential at a point by the relation

$$n_1 \bar{E}_s = \int n_s dE_s \quad (3a)$$

where n_s is the number of electrons accepted at the source potential E_s in a small time interval during which a total of n_1 electrons are accepted. It may be presumed that the relation of n_s to E_s is of the nature of a Maxwell-Boltzmann distribution function. The integral is applied over all cytochrome enzymes that accept electrons.

Similar definitions apply to the mean terminal potential, \bar{E}_t , which is averaged for electrons being accepted by oxygen or other terminal acceptors. Normally the average is to be taken over all electrons leaving the system via cytochrome oxidase. Thus

$$n_2 \bar{E}_t = \int n_t dE_t \quad (3b)$$

where n_t is the number of electrons donated at the terminal potential E_t by ferro-cytochrome oxidase to an acceptor during a short time interval in which n_2 electrons are transferred to the acceptor. E_t is assumed to vary from point to point over the terminal cytochrome and from instant to instant. The integration is over all electrons leaving the system.

The mean difference of cytochrome potential is accordingly given by

$$\bar{E}_{st} = \bar{E}_t - \bar{E}_s \quad (4)$$

and is determined by the mean potentials at which electrons enter and leave the system. Normally it depends, for example, on the ratio of electrons accepted by cytochromes b and c, and on the average ratio of ferri- to ferro-cytochrome oxidase.

In the experiments on the synovial membrane, it has appeared permissible to assume in most cases that the inhibitors and activators affect metabolism only on one side of the membrane, the joint cavity side where the solutions are introduced.

On the other side, under the conditions of the experiments, the cytochrome potentials are evidently not seriously changed. Treating the membrane as two boundaries without transference, the membrane potential E_m can be represented by

$$\bar{E}_m = \bar{E}_{st2} - \bar{E}_{st1} \quad (5)$$

where \bar{E}_{st2} , the mean difference of cytochrome potential on the unaffected side of the membrane, can be regarded as a constant. \bar{E}_{st1} is the mean difference of cytochrome potential at the inner boundary of the membrane. The equation includes only the large metabolic terms in the potentials, neglecting the much smaller diffusion potentials. Variations of the membrane potential due to experimental factors are

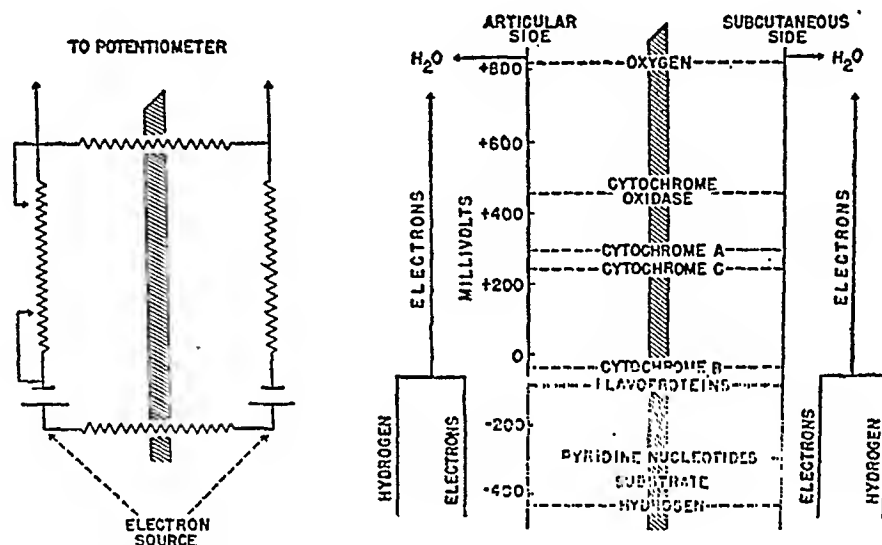


Fig. 1. DIAGRAM ILLUSTRATING ELECTRICAL PROPERTIES of the synovial membrane in relation to some of the enzyme systems. Approximate standard potentials of enzymes arranged in vertical series with hydrogen and electron transport proceeding from low to high potentials. Electrons are separated from hydrogen at a mean potential, defined as the mean source potential. They are separated from the cytochrome system normally by oxygen at a higher level, defined as the mean terminal potential. The mean difference of potential, \bar{E}_{st} , depends on the nature of activation or inhibition at the source or terminal. One of the two approximately equal and opposite differences of potential can be varied experimentally by treating one side of the membrane with metabolic inhibitors or activators. The horizontal resistances in the electrical diagram denote electrolytic resistance which depends on ionic transference. The greater this resistance, the more nearly the limiting case of the boundary without transference is approached.

thus attributed to changes of \bar{E}_{st1} . The nature of these changes are now to be considered, bearing in mind the fact that equation 5 is applicable only when transference is small.

In figure 1, the redox potentials of several respiratory enzymes are represented in a manner which indicates their functioning in hydrogen or electron transport systems. All enzymes and carriers functioning at potentials in the range from that of cytochrome *b* down to -420 mv., the potential of the hydrogen electrode at pH 7, are involved in processes of hydrogen transport, consisting of successive hydrogenations and dehydrogenations. Such processes depend on the exchange of atomic or free radical hydrogen, which can be regarded as an electron plus a proton. The

reversible redox potentials of such enzymes as the pyridine nucleotides (coenzymes) and flavo-proteins depend on pH according to the thermodynamic equations derived for hydrogenations by Clark (9) and by Michaelis (10) and others. The same is true of the potentials of metabolites and carriers involved in dehydrogenations. These hydrogen transport systems, predominantly soluble components in the liquid phase, have long been regarded as constituents of the 'accessory respiratory systems' (11). They are distinguished from the cytochrome system, which is involved only in electron transport rather than hydrogen transport, and which occurs predominantly in insoluble structures. The two processes are to be distinguished, in the interpretation of results, by some correlated facts. The separation of electrons from protons (hydrogen ions), which occurs at the cytochrome source potential level, leads to differences of potential arising from the independent mobility of electrons and hydrogen ions, the latter combining with various buffers and diffusing slowly in the liquid phase. The electrons, on the contrary, are conducted rapidly along the solid structures containing the insoluble cytochromes.

In addition, the cytochromes are distinguished from the hydrogen transport systems by the fact that their standard redox potentials are independent of the pH , as is to be predicted for redox systems that react only by electron exchange (9, 12). For these reasons the cytochrome system can be conceived as an electron conductor which carries a steady current and in which there is normally maintained a steady potential gradient. Oxidoreductions occurring in the auxiliary system involve the simultaneous transport of electrons and protons, and lead to no potential differences or currents. The reactions involve essentially only diffusion and molecular collisions resulting only in transport of hydrogen. Figure 1 illustrates the operation of two parallel circuits corresponding to two oppositely directed metabolic gradients in the membrane, indicating how the source and terminal potentials may be varied by inhibitors or activators.

The mean cytochrome potential difference, $\bar{E}_{s,t}$, represents a difference of energy levels between electrons entering and leaving the system. The application of this concept to a few cases is illustrated by the energy-level diagram of figure 2. The levels for cytochromes *b* and *c* are taken from the values determined by Ball: cytochrome *b*, $E^0 = -0.040$ volts and cytochrome *c*, $E^0 = 0.27$ volts (13). Other values for cytochrome *c* are 0.254 and 0.272 (14, 15). The value of E^0 for cytochrome oxidase has not been reliably determined, but Ball estimates it to be of the order of $+0.500$ volts (12). That it is somewhere between $+0.290$ volts, the value of E^0 for cytochrome *a* (13), and $+0.800$ volts, the value for oxygen, is all that is known with certainty. Accepting provisionally the above values for cytochrome *b* and cytochrome oxidase, it follows that $\bar{E}_{s,t} = 540$ mv., if \bar{E}_s and \bar{E}_t are set equal to the standard potentials of cytochrome *b* and cytochrome oxidase. This is equivalent to the assumption that the mean source potential corresponds to that of half oxidized cytochrome *b*, and that the mean terminal potential corresponds to that of half-oxidized cytochrome oxidase. If an appreciable fraction of the electrons enter the conducting system at the cytochrome *c* level, $\bar{E}_{s,t}$ would have a smaller value, because the source potential would be shifted toward the terminal potential. Figure 2 represents the potential levels in the cytochrome system, corresponding to cytochromes

b and *c*, and cytochrome oxidase. Each of the components is represented in its standard state (half oxidized, half reduced), in an oxidized state (90%), and in reduced state (90%). The displacement of each of these is 60 mv. from the standard, positive for the oxidized level, and negative for the reduced. This highly simplified schema permits one to visualize roughly the effects of metabolic inhibitors or activators. The diagram represents 3 substances with entirely different effects, each compared with the normal difference in energy level. The inhibitory effect of cyanide on cellular respiration has been explained as resulting from its combination as an iron complex with cytochrome oxidase (4). The cyanide-enzyme complex is no longer able to transfer electrons from the other cytochromes to oxygen. Accordingly, on the energy level diagram, an arrow from the cytochrome *b* level to the cytochrome *c*

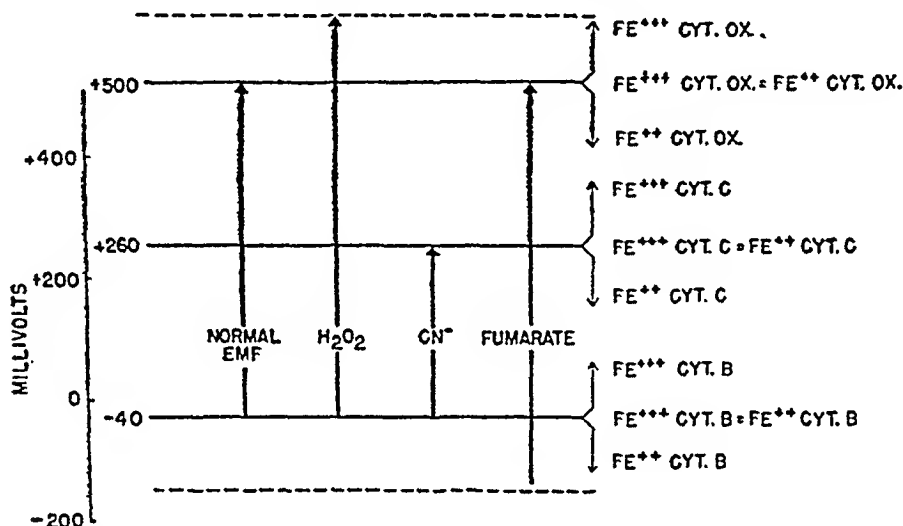


Fig. 2. ENERGY LEVELS IN THE CYTOCHROME SYSTEM, based on values for the standard potentials of the cytochrome components. The length of each arrow is proportional to \bar{E}_{st} , the mean difference of potential between source and terminal. The values illustrated are for cyanide, hydrogen peroxide, sodium fumarate and the normal untreated tissue.

level represents the mean potential difference, \bar{E}_{st} , which amounts to 300 mv., compared with the normal difference of 540. According to equation 5, $E_n = 540 - 300$, or 240 mv., a value which is uncertain because of the uncertainty of E^0 for cytochrome oxidase. The observed average (table 1) is 180 mv. The calculated value, however, should be approached only for an ideal boundary without transference. The actual system more probably corresponds to a boundary with partial transference. Similar considerations apply to all other cases.

The other examples shown on the energy level diagram (fig. 2) are explained as follows. Hydrogen peroxide, as an electron acceptor or as a source of molecular oxygen formed by the action of catalase, oxidizes cytochrome oxidase, increases the mean terminal potential, \bar{E}_t , and also the value of \bar{E}_{st} . According to equation 5 this leads to a negative value of the membrane potential, in contrast with the effects of cytochrome oxidase inhibitors. Fumarate ions stimulate the rate of aerobic metabolism (16, 17). According to Szent-Györgyi, the effect occurs at the succinic dehydrogenase level, where electrons are transferred to ferri-cytochrome *b*, and hydrogen

ions are formed from the substrate. On the energy level diagram, the effect can be represented by indicating the source potential as that of partially reduced cytochrome *b*, the terminal potential remaining at or near its normal level. The use of the diagram in such cases is qualitative and descriptive only as a first approximation. Quite probably in any actual process both source and terminal potentials vary.

TABLE 1. CYTOCHROME OXIDASE INHIBITORS AND METHYLENE BLUE

INHIBITOR	NO. EXPER.	CONC. ¹	E - E _{NaCl}	CHARACTERISTICS
		mol/l.	av. in mv.	
Na ₂ S	8	0.015	+350	Maximal initially. Drifts negative. Becomes negative after replacement with isotonic NaCl.
Na ₂ S + leuco methylene blue (0.01%)	6	0.015	+350	Stabilized by dye.
Na ₂ S + oxidized methylene blue (0.1%)	6	0.015 ²	+270	Stabilized by dye.
NaCN	6	0.03	+180	Stable maximum after short exposure. Reversible with NaCl (2-3 washings).
NaCN + oxidized methylene blue	4	0.03	+180	
Na Azide	4	0.03	+60	Reversible with NaCl.
Hydroxylamine hydrochloride	4	0.03	+80	" " "
Methylene blue (0.01%)	6		+6	" " "

¹ Minimum concentration required to develop given potential. All solutions made isotonic with NaCl.

² Solution prepared by aerating leuco dye + Na₂S.

INTERPRETATION OF RESULTS

In the following paragraphs, the experimental results of tables 1 to 5 are discussed with reference to the known effects of the substances as inhibitors or activators. As a basis of discussion, the variations of potential will be represented by *equation 5* and by the energy level diagram of figure 2. The observed potentials should correspond to those represented in the figure only in the case of boundaries without transference. All other potentials require correction due to ionic conductance across the membrane.

Cytochrome Oxidase Inhibitors (Table 1). A comparison of the effects of sodium cyanide and sodium sulphide at various concentrations is shown in figure 3. The effects of methylene blue on both inhibitors are also shown. The results in table 1 include also the effects of azide and hydroxylamine. All these inhibitors have been recognized to be specific for cytochrome oxidase, preventing the passage of electrons from the other cytochrome components to oxygen (4, 12). It is important to observe with these inhibitors that the maximal effects on the potential occur at higher concentrations (0.015-0.03 M) than are necessary to cause practically complete inhibition of

cellular respiration (0.001 M). At these lower concentrations the membrane potential with cyanide or sulphide is of the order of 20 or 30 mv., compared with the maximal of 150 to 400 mv. developed at higher concentrations. There is, accordingly, no direct proportionality between the inhibition of oxygen consumption and the membrane potential produced by one of these substances. Therefore, $\bar{E}_{s,t}$ does not necessarily indicate the rate of flow of electrons over the cytochromes. This rate corresponds to n_2 of equation 3b, which is the number of electrons yielded to oxygen per unit of time. As n_2 approaches zero for complete inhibition at low concentration, E_m shows no corresponding approach to a maximum (fig. 3). This fact can be expressed by the statement that the ratio $\bar{E}_{s,t}/n_2$ becomes maximal in the presence of the inhibitor. As this is the ratio of a potential difference to a current, it can be taken to express resistance in the cytochrome system. Thus the effect of the inhibitor can be repre-

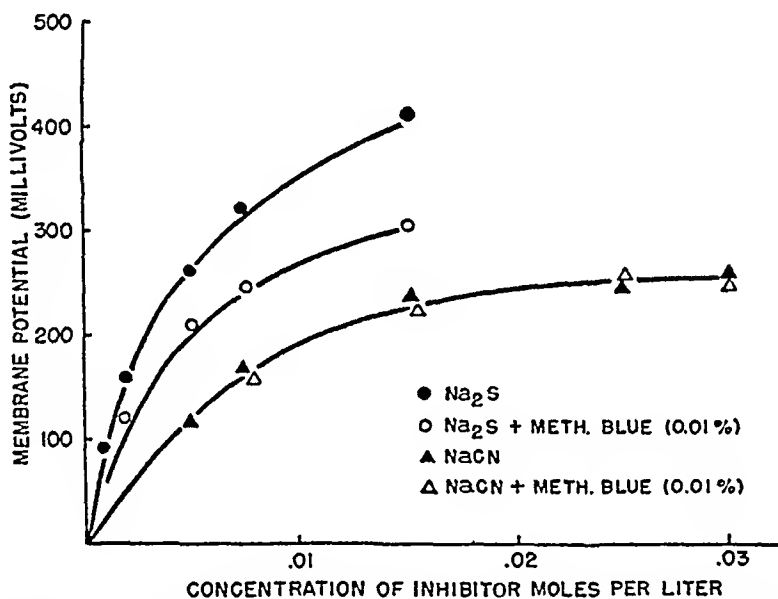


Fig. 3. RELATION BETWEEN MEMBRANE POTENTIAL and concentration of inhibitor for cyanide and sulphide inhibition. Results given both in presence of methylene blue and in its absence. All solutions made isotonic with NaCl.

sented as a large increase of this resistance. For complete inhibition of tissue respiration, $\bar{E}_{s,t}$ is essentially a static difference of potential; for normal tissue respiration it is a difference of potential maintained with a constant electron current, as long as the metabolic rate is constant.

To describe, by means of the energy level diagram, the effect of NaCN, the terminal potential \bar{E}_t has been represented as dropping from its assumed normal level of 500 mv. to the cytochrome *c* level, 260 mv. Ball has favored the hypothesis that cytochrome oxidase reacts with its inhibitors to form complexes with lower standard potentials than that of the free enzyme (12). The potential of each complex is specific. For example that of the azide complex is considered higher than that of the cyanide complex. The experimental results on membrane potentials can be interpreted from this point of view, the maximum potential obtained with cyanide being found roughly to correspond to a terminal potential at the cytochrome *c* level. As

previously explained, allowance must be made for the effects of transference on the observed potentials.

Typically, the effect of sulphide on the membrane potential is considerably greater than that of cyanide. In the presence of oxidized methylene blue, the potential of sulphide tends to approach that of cyanide at the same concentration. Methylene blue has little or no effect on the cyanide potential curve (fig. 3). The effects can be interpreted in the following way. In the presence of sulphide, \bar{E}_t corresponds to a level for greatly reduced cytochrome *c*. Oxidized methylene blue tends to re-oxidize the reduced cytochrome *c* to a level nearer that occurring in cyanide inhibition. An enzyme, cytochrome peroxidase, which catalyzes the oxidation of cytochrome *c* by hydrogen peroxide has been described (18). Methylene blue, as an intermediary carrier from metabolites to oxygen, forms hydrogen peroxide. This auxiliary pathway does not appreciably change the terminal potential in the case of cyanide inhibition, presumably because the cytochrome *c* is at a more oxidized level. The cyto-

TABLE 2. EFFECTS OF THIOLS

THIOL	NO. EXPER.	CONC. ¹	E - E _{NaCl}	CHARACTERISTICS
		mol./l.	av. in mv.	
Thiourea	8	0.03	+220	Maximum after 3-5 min. Reversible with NaCl.
Urea	4	0.03	+4	Reversible
Thiouracil	6	0.01 ²	+50	Maximum after 3-5 min. Reversible.
Glutathione	5	0.01	-10	Reversible.
Sodium thioglycolate	8	0.03	+170	Maximum after 3-5 min. Reversible.

¹ Minimum concentration required to develop given potential. All solutions made isotonic with NaCl.

² Limited by solubility.

chrome peroxidase system is a possible mechanism for maintaining the cytochrome *c* potential level, but one which is not sufficient to compete with the strong reducing action of the hydrosulphide ion. Wieland and Sutter (19) have demonstrated the inactivation of peroxidase by sulphide. Thiols, such as thioglycolic acid, produce high positive membrane potentials, which also appear to be explicable as reducing action at the cytochrome *c* level.

Effects of Thiols (Table 2). With the exception of glutathione, none of the thiols included in table 2 is a natural constituent of biological fluids. Urea is included in the table for comparison with thiourea. Thioglycolate, thiourea and thiouracil all show high positive potentials. All are metabolic inhibitors. Glutathione, however, shows a small negative potential. The effects of the thiol inhibitors can be interpreted as the transport of electrons to the cytochrome *c* level rather than to cytochrome *b*. The inhibitors thus compete with normally active enzymes and carriers, carrying the electrons to a higher source potential. \bar{E}_t then tends to approach the cytochrome *c* potential, which is approximately 300 mv. higher than that of cytochrome *b*. Observed potentials with thiourea and thioglycolate are often greater

than 200 mv. Thiouracil shows smaller effects, but is only slightly soluble (0.01 M). Partial transference again must be considered to affect the results. As the first approximation, however, the results can be expressed by means of equation 5 or the energy level diagram where the source potential, \bar{E}_s , is taken as more positive than normal. This type of inhibition differs from the cyanide type in which the terminal potential is taken as less than normal. Both effects result in high positive observed potentials.

Heavy Metals and Other Sulfhydryl Inhibitors (Table 3). Heavy metals influence metabolism in numerous ways. They combine with the sulfhydryl groups both of enzymes and of thiols such as glutathione (20,21). They are very active catalysts for oxidoreductions involving thiols, and accordingly affect the pathways of the respiratory systems. In addition, they are involved in other types of complex systems,

TABLE 3. HEAVY METALS AND OTHER SULFHYDRYL INHIBITORS

INHIBITOR	NO. EXPER.	CONC. ¹	E - E _{NaCl}	CHARACTERISTICS
		mol./l.	av. in mv.	
CuCl ₂	12	0.00075	+180	Stable for several min. Difficult to reverse with NaCl. Reversible with 0.4% glutathione in NaCl.
FeCl ₃	8	0.0075	+220	Initial maxima which drift toward negative. Reversible with several washings in NaCl. Easily reversed with 0.015 M Na pyrophosphate.
HgCl ₂	6	0.00075	+180	Difficultly reversed. Reversible with glutathione (0.4%)
p chlormercuribenzoate	6	0.003	+50	Maximum in 4-5 min. Reversible with NaCl.
Na o-iodibenzoate	6	0.00075	+100	Maximum in 4-5 min. Reversible with NaCl.
Na iodoacetate	5	0.015	+100	Develops rapidly. Reversible with NaCl.

¹ Minimum concentration necessary to develop given potential. All solutions made isotonic with NaCl.

such as the iron enzymes, catalase, peroxidase and the cytochromes, as well as the copper containing protein complexes, hemocuprein and hepatocuprein (22). Further indications of the complexity of their behavior are given by the results of Ames, Ziegenhagen and Elvehjem (23) who have found that various inhibitors are specific for iron and copper, as well as for various respiratory enzymes. Substances that inhibit iron retard the oxidation of ascorbic acid, copper inhibitors impede the oxidation of glutathione, while the oxidation of succinate depends on both. Cytochrome *c* catalyzes the oxidation of these substrates, the oxidation of succinate requiring the enzyme succinic dehydrogenase (4). These facts are in agreement with the effects of ferric and cupric ions on the membrane potentials, which are of the order of 200 mv. at the most effective concentrations. The metals, by catalyzing the oxidation of one or more of the systems, glutathione, ascorbic acid, succinic acid, establish an auxiliary pathway by which electrons are separated from protons at

the cytochrome *c* level, rather than at cytochrome *b*. \bar{E}_s is thus increased, and \bar{E}_{st} decreased. This effect would be sufficient to explain the result, and the similarity with the effects of the thiol inhibitors. Actually, however, the metals probably react in several additional ways. According to Keilin and Hartree (24), copper oxidizes all the cytochrome components. Also, the heavy metals act as sulfhydryl inhibitors, decreasing the rate of glycolysis and other enzymatic dehydrogenations at potentials below the cytochrome *b* level.

The sulfhydryl inhibitors, p-chloromercuribenzoate, o-iodobenzoate and iodoacetate act more specifically on the latter processes (25). All of them produce positive membrane potentials, which may be interpreted as caused by an increased value of the mean source potential, \bar{E}_s . This would be a direct result of slowing the rate at which electrons are transferred to cytochrome *b*.

Succinic Dehydrogenase Inhibitors, Activators and Substrate (Table 4). Malonate is an inhibitor which acts with some specificity on the enzyme succinic dehydrogenase (17, 26). When introduced into the joint cavity, it produces a positive potential of about 50 mv., which, however, soon drifts toward zero. The positive potential is easily explained as the result of an increased potential due to inhibition of electron transport through succinic dehydrogenase. The drift could possibly be explained as a subsequent oxidation of the terminal cytochrome oxidase with an increase of \bar{E}_t . Again it is evident that there is no necessary direct connection between the metabolic rate and the membrane potential. With strong inhibition, \bar{E}_{st} is essentially a static difference of potential, and may have a value very close to normal.

Sodium fumarate stimulates metabolism through the succinic dehydrogenase system (16, 17). It produces a negative membrane potential of about 30 millivolts (table 4). This persists after numerous washings with NaCl, indicating that the fumarate is firmly bound to the enzyme. In this case, the source potential is more negative than normally because of the greater rate at which cytochrome *b* is reduced by the substrate-enzyme system. The source potential, \bar{E}_s , evidently depends on the rates at which electrons are added to and removed from cytochrome *b*. It represents essentially a balance between the rates of oxidoreductions in the cytochromes and in the dehydrogenation processes which supply electrons to cytochrome *b*. Sodium succinate yields a negative potential of the order of 20 mv., which drifts toward zero. It is ineffective at low concentrations, and seems to behave simply as a substrate rather than as a coenzyme.

Electron Acceptors (Table 5). Iodine and hydrogen peroxide are placed in this group because their effects on the potential appear to indicate that initially at least they oxidize at the cytochrome oxidase level. The instability of the initial negative potentials, which drift toward positive, may indicate a slower tendency to oxidize the other cytochrome components. The behavior of iodine differs in solutions containing iodide ions compared with those that contain only chloride ions. In the latter solutions the initial potentials are of the order of minus 50 mv., but they rapidly drift beyond zero and become quite positive. In solution with iodide ions, iodine always yields positive potentials. The value depends on the concentration of the iodide ions. In the presence of 0.15 M NaI, the potential given by 0.0015 M iodine

is approximately 200 mv. At lower concentrations of iodide ion, the solutions being made isotonic with NaCl, the potentials are lower, but continuously drift positive. Isotonic NaI itself yields a positive potential of about 25 mv. (1).

The above facts suggest that iodide and iodine act in an auxiliary hydrogen transport system which is capable of carrying electrons to the cytochrome *c* level. The initial effect of iodine appears to be that of an electron acceptor at the cytochrome oxidase level, producing increases of \bar{E}_t and \bar{E}_{st} . As soon as traces of iodide ion are formed the carrier system operates, and the secondary effect is to increase \bar{E}_s . Accordingly, \bar{E}_{st} decreases, and the observed potential becomes positive. Since molecular iodine is capable of oxidizing glutathione, this may possibly be part

TABLE 4. INHIBITORS AND ACTIVATORS OF SUCCINIC DEHYDROGENASE

REAGENT	NO. EXPER.	CONC. ¹	E - E _{NaCl}	CHARACTERISTICS
		<i>mol./l.</i>	<i>av. in mv.</i>	
Na malonate	8	0.01	+50	Unstable initial maximum. Drifts toward zero.
Na fumarate	8	0.015	-30	Immediate effect which persists after several washings with NaCl.
Na succinate	6	0.075	-20	Unstable initial potential. Drifts toward zero.

¹ All solutions made isotonic with NaCl.

TABLE 5. ELECTRON ACCEPTORS

REAGENT	NO. EXPER.	CONC.	E - E _{NaCl}	CHARACTERISTICS
		<i>mol./l.</i>	<i>av. in mv.</i>	
I ₂ in 0.15, M NaCl	12	0.0015	-50	Initial negative potential which rapidly drifts positive.
I ₂ in 0.15 M NaI.	12	0.0015	+200	Partially reversed with NaCl. Potential maintained after washing with isotonic NaI.
H ₂ O ₂ in 0.15 M NaCl	8	0.01	-70	Drifts positive slowly. Reversible with NaCl.

of the system involved. The mechanism might also account for the potentials observed with isotonic iodide, which are abnormally positive compared with those of chloride and bromide. Tissues oxidize iodide ion by means of peroxidase (27). Thus the carrier system responsible for the positive potential would be formed either from the ion or from molecular iodine. This mechanism results in inhibition similar to that given by the heavy metal-thiol systems, which also yield positive potentials. All are explicable as oxidations of cytochrome *b* by transport of electrons and protons to higher cytochrome levels through the action of auxiliary hydrogen transport systems.

Hydrogen peroxide produces an initial negative potential, which ultimately drifts toward zero. Its initial effect is to increase \bar{E}_{st} by oxidizing cytochrome oxidase (fig. 2). The secondary positive drift is possibly the subsequent effect of oxi-

dations of the other cytochrome components, possibly through cytochrome peroxidase (18).

Relation to Polarization Potentials. An earlier investigation of the electrochemical properties of the synovialis included experiments on the potentials produced by polarization of the membrane brought about by application of an external electromotive force (1). For voltages above a certain threshold applied for two or more minutes, the polarization potentials that were produced were often noted to be fairly stable for as long as 5 minutes, depending on the polarizing current and the time it had been applied. The level of the stable polarization potential was nearly always observed to be between 150 and 200 mv. The sign was determined by the direction of the applied current, either positive or negative. The magnitude was as a first approximation independent of the applied voltage as long as this exceeded the threshold level. Reconsideration of these facts suggests that they are explicable in terms of the mechanism proposed in the theoretical section of this paper. An externally applied field necessarily induces a displacement current of electrons in the cytochrome system as well as migration of ions. Electrons would be displaced toward the anode, reducing ferri-cytochrome oxidase to the ferro-enzyme on the anodic side of the membrane. Eventually, the terminal cytochrome potential would be reduced to the cytochrome *c* level, where presumably it stabilizes because the concentration of the *c* component is much greater than that of the others. The galvanically induced reduction of cytochrome oxidase that is postulated leads to inhibition on the anodic side of the membrane. The type of inhibition is that in which the mean terminal potential, \bar{E}_t , decreases, dropping toward the cytochrome *c* level. With removal of the applied current, this displaced potential would manifest itself as a polarization potential, which persists as long as the cytochrome oxidase is reduced. Thus the length of time during which the polarization potential is stable depends on the quantity of cytochrome *c* reduced during the period of polarization. This agrees with the experimental evidence which shows that it is the time of stability rather than the potential level which is proportional to the quantity of current transferred. The magnitude of the potential, 150 to 200 mv., also in in agreement with the postulate, for it agrees fairly well with that observed with cyanide as an inhibitor, in which case the terminal potential is regarded as dropping to the cytochrome *c* level. The above considerations indicate that there is a reciprocal relation between metabolism and potentials; metabolism may depend on induced galvanic currents, and conversely potential gradients in tissues depend on metabolic rates and pathways.

DISCUSSION

The relation of polarization potentials, and bioelectric fields in general, to growth and metabolism have been discussed in a recent monograph by Lund (28). As experimental material, vegetable preparations were most extensively studied. The results show numerous interrelations between the bioelectric fields, polarization in applied fields and metabolism as influenced by inhibitors. The magnitudes of the potentials quite clearly are beyond those predicted for ionic diffusion potentials. The relation of membrane potentials to metabolism has been a controversial sub-

ject (29). Although earlier work has shown that metabolic inhibitors produce high potentials, the origin of the potential has been a source of disagreement. A magnitude of the order of 100 mv. would require an ionic concentration gradient of about 100 to 1 across a membrane with very high selectivity toward one of the ions. Under the conditions of these experiments, no such ratio could possibly develop. The earlier results on metabolically inactive ions indicate very little selectivity by the synovial membrane (1).

In accounting for the potentials as originating in tissue oxidoreductions the difficulty consisted in accounting for an electron transfer mechanism (29).

In boundaries with transference, high potentials resulting from oxidoreductions are theoretically impossible. The cytochrome system, conceived as an electron conductor, resolves this difficulty by providing a natural boundary with partial transference. The system can be conceived as oriented toward the oxygen supply of the tissues, with the *b* and *c* components taking up electrons at a low potential and conducting them rapidly to the oxidase at a high potential (5). The normal potential difference in the system is of the order of 500 mv. Because of symmetrical orientation, the normal membrane potential is the resultant of two nearly equal and opposite vectors. It therefore is of a small order of magnitude. Inhibitors or activators can selectively act on one of the component vectors, and produce high potentials. Polarization in an external field can lead to a similar result. Both effects are readily accounted for by the assumed mechanism.

SUMMARY

The effects of various types of inhibitors and activators on the potential of the synovialis in dogs have been determined. Among the agents studied were cytochrome inhibitors, sulfhydryl inhibitors, thiols and inhibitors of succinic dehydrogenase.

Theoretical considerations based on the properties of artificial boundaries with and without transference have been presented to facilitate the interpretation of the results. On the basis of the theoretical considerations, the observed potentials have been related to the known metabolic effects of the inhibitors and activators. The effects are referred to mean differences of potential between the source and terminal in the cytochrome system. Inhibitors, in general, by decreasing this difference of potential on one side of the membrane, produce a high positive potential across the membrane. Activators show the opposite effect. The magnitude and sign of the potential can be related to the effects on enzymes, coenzymes or carriers. The results are discussed in connection with an energy level diagram giving the standard potentials of the cytochrome components.

It is shown that a galvanic current can lead to inhibition on the anodic side of the membrane, and that the relation between metabolism and the electric field is reciprocal.

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CORRELATION BETWEEN SIGNS OF TOXICITY AND CHOLINESTERASE LEVEL OF BRAIN AND BLOOD DURING RECOVERY FROM DI-ISOPROPYL FLUOROPHOSPHATE (DFP) POISONING

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SEVERAL investigators have reported that for the brief period immediately following the injection of di-isopropyl fluorophosphate (1, 2), a close relationship exists between the severity of toxic signs and the degree of depression of brain cholinesterase activity, a relationship which with one exception (3), has not been considered coincidental. However, during the more prolonged recovery from DFP the relationship between toxic signs and brain cholinesterase activity has not been explored. Yet studies of Grob, *et al.* (4) on man indicate the necessity of such data in explaining the behavioral changes. These investigators found that following recovery from a large dose of DFP, clinical signs of toxicity disappeared within 48 hours while the red blood cell cholinesterase activity remained stationary during that time. Erythrocyte cholinesterase activity has been considered a reflection of brain cholinesterase activity because of the parallelism between the depression of the cholinesterase activity of erythrocytes and that of brain by DFP *in vitro* (3) and *in vivo* in the acute stages of DFP poisoning. One may, therefore, conclude from the experiments of Grob *et al.* (4) that brain cholinesterase remains stationary during the 48-hour period while the toxic signs disappear. In other words, the brain becomes acclimatized to function relatively normally at a level of tissue cholinesterase considerably below the normal of optimal range. Another explanation of this experiment would be that the brain cholinesterase regenerates more rapidly than the erythrocyte cholinesterase. The authors questioned the latter possibility because Mazur and Bodansky (3) and Koelle and Gilman (1) had reported that in the rabbit and rat the red blood cell cholinesterase returns to normal before the brain cholinesterase activity. Nevertheless the data of Koelle and Gilman (1) on the rat suggests that during a limited period immediately following the injection of DFP the rate of regeneration of erythrocyte cholinesterase is slower than brain cholinesterase. In order to investigate more completely the rates of regeneration of the cholinesterase of brain and blood and, in that way, study the relationship between cholinesterase activity and toxic signs the following experiments were undertaken.

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METHOD

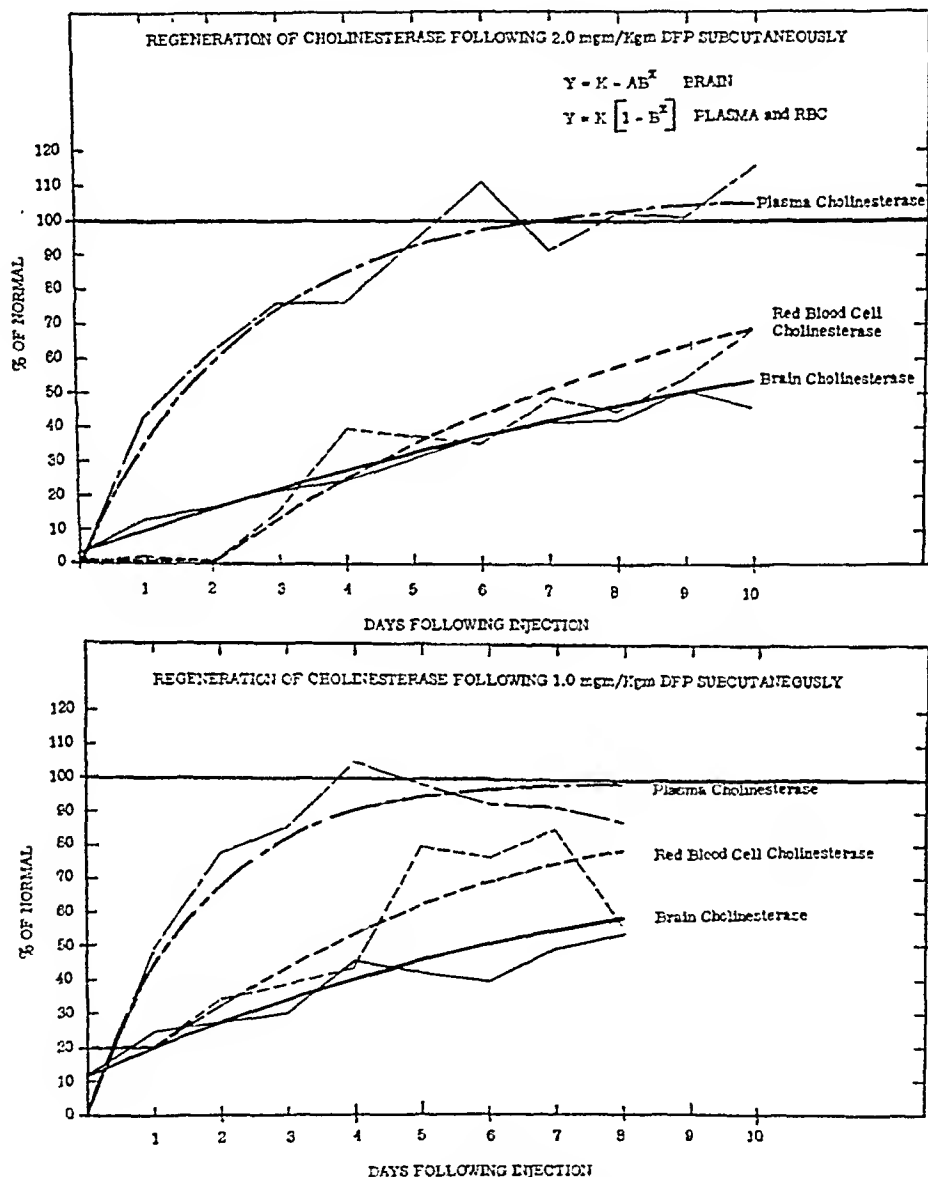
A total of 94 adult male rats weighing between 125 and 175 grams were used. The animals were divided into groups of 20 to 25 animals and each group was studied in the following manner. All rats were injected subcutaneously either with 1.0 or 2.0 mg/kg. DFP in saline, every member of a given group receiving the same dose. One hour following the injection, the rats in the group were observed for toxic signs. Subsequently, the animals were inspected daily. The manifest signs of toxicity at each inspection time appeared quite uniform within each group. Toxic death occurred chiefly during the first hour when approximately 25 per cent of the rats died with the 2.0-mg. dose and less than 10 per cent succumbed with the 1.0-mg. dose. Only animals with longer survival periods were examined for behavioral and biochemical changes.

At each observation two rats were selected at random from the group. Each of these rats was carefully studied noting all behavioral signs of toxicity. In evaluating the signs of toxicity, the following criteria were used: Animals were graded 'severe' where they displayed evidences of grave toxicity such as marked spontaneous trembling of the body, great hyperreactivity upon tapping the spine, muscular weakness of varying degrees culminating in paralyses, and excessive salivation. Rats graded as of 'moderate' toxicity exhibited manifestations including intermittent periods of trembling, marked motor restlessness, moderate hyperreactivity upon tapping the spine and spontaneous fasciculations of the muscles in the flanks. Animals rated as exhibiting 'slight' toxicity showed signs such as transient fasciculations while standing erect on their hind legs and motor restlessness. Animals that were free of overt toxic signs were considered 'normal'. Members of each group were studied until the remaining members became free of toxic signs. Then these rats were killed and the cholinesterase activity of the whole brain, packed erythrocytes and plasma were determined. The brain cholinesterase was determined as described in previous work (2). The erythrocytes and plasma cholinesterase were also measured manometrically. The erythrocytes were not washed but carefully separated from the plasma after prolonged centrifugation in a hematocrit tube. It was found that better agreement between samples obtained from control rats could be found without washing although the mean activity was not significantly altered. A further correction was necessary in determining the cholinesterase activity of the erythrocytes. Because their anaerobic glycolysis produced significant amounts of CO_2 , a sample without acetylcholine was run simultaneously with each erythrocyte determination and the figure so obtained subsequently subtracted. Control values for the brain and blood cholinesterase activity were obtained from 17 normal rats.

RESULTS

Figure 1 indicates regeneration of cholinesterase in the brain, plasma and erythrocytes following a dose of 2.0 mg/kg. of DFP. In this and subsequent charts the broken line is drawn through the determined points. It was found that these data could be best described mathematically in all cases by the equation $Y = K - AB^x$

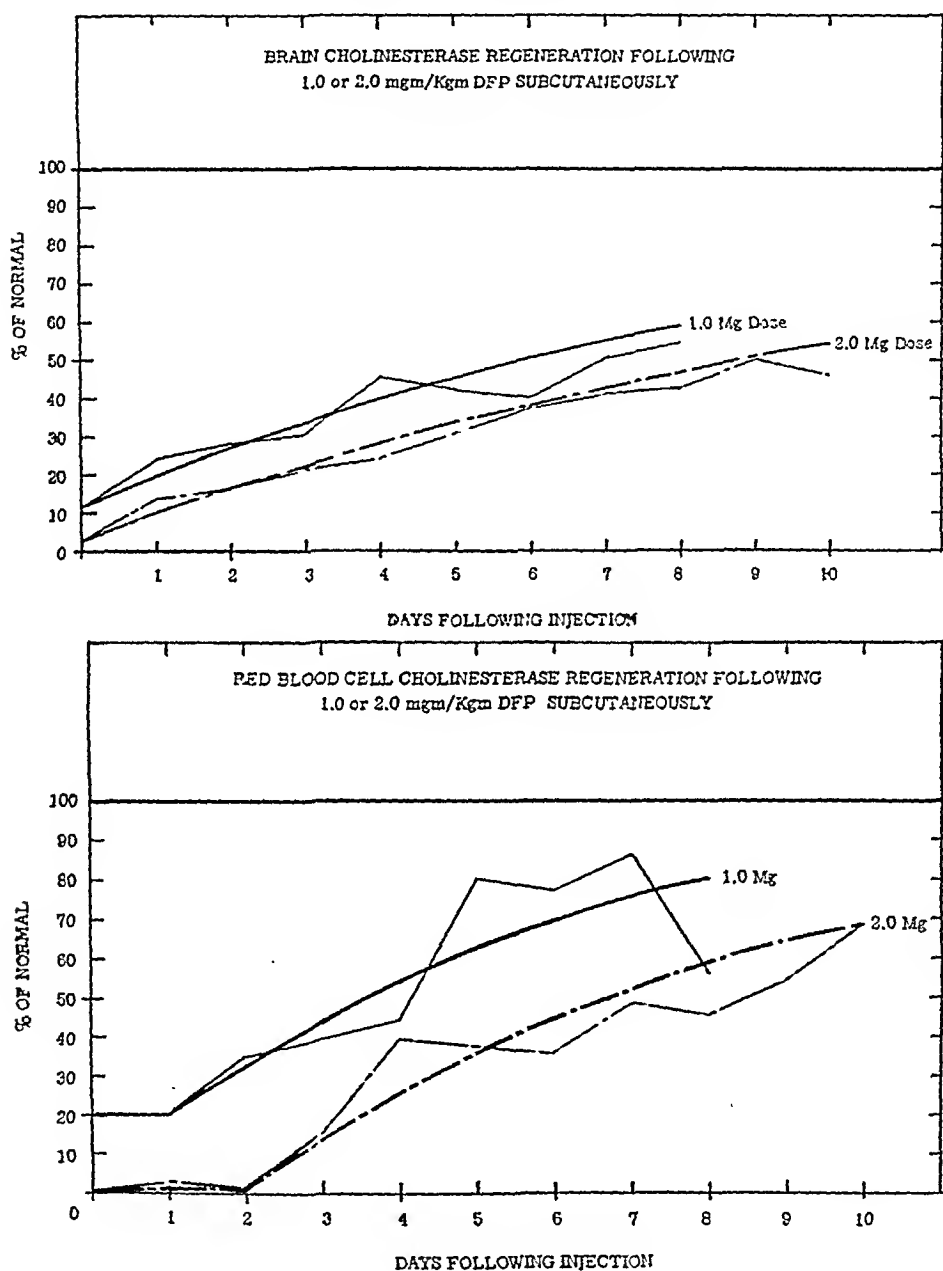
for the brain and $Y = K(1 - B^x)$ for the plasma and erythrocytes. These equations are represented by the smooth curves that describe the regeneration plasma, red blood cell, and brain cholinesterase respectively. The nonspecific plasma cholinesterase regenerated most rapidly and reached normal within 7 days. On the other



Figs. 1 (upper) and 2 (lower)

hand during the first 48 hours the specific erythrocyte cholinesterase activity remained almost at zero while the brain cholinesterase increased more than threefold. Subsequently the rate of red blood cell cholinesterase increased and the red blood cell line in the figure can be seen crossing the brain line. A similar study was performed with rats injected with 1.0 mg/kg. DFP as indicated in figure 2. Again a lag in the regeneration of red blood cell cholinesterase activity is observed. In this instance

the lag was only 24 hours during which time the brain cholinesterase was almost doubled. The similarity in the regeneration patterns when either 1.0 or 2.0 mg/kg. DFP was injected is further emphasized in figure 3 where the brain cholinesterase for each dose is plotted. The parallel in the trend lines is apparent. Similarly in



Figs. 3 (upper) and 4 (lower)

figure 4 the erythrocytes cholinesterase activity for both the 1.0 and 2.0 mg/kg.-dose are indicated. In each case one observed first a lag and then parallel lines of regeneration.

Table 1 indicates the relationship between the severity of the clinical signs and the levels of the various cholinesterase activities in the rats injected with 2.0 mg/kg.

The mean time of appearance in days of each of these symptom groups is likewise indicated. One notices the steady increase in the means of the brain cholinesterase activity and the improvement in clinical signs.

The scatter of the brain cholinesterase corresponding to each clinical category is not wide as can be seen from the low standard error of the mean. Furthermore, by the *t*-test it has been found that each of the mean brain cholinesterase activities is significantly different from every other (table 2). This indicates that the classification is valid and that each category is sharply delineated from the next. In the case

TABLE 1. RELATIONSHIP OF SIGNS OF TOXICITY TO CHOLINESTERASE ACTIVITY FOLLOWING 2.0 MG/KG.

SYMPTOMS	BRAIN CHOLINESTERASE % NORMAL	RBC CHOLINESTERASE % NORMAL	PLASMA CHOLINESTERASE % NORMAL	APPEAR- ANCE, TIME-DAYS
Severe.....	2.9 ± .32 ¹	0	0	.042
Moderate.....	17.9 ± 2.0	10.4 ± 3.9	59.8 ± 10.8	2.35
Slight.....	35.9 ± 2.5	37.5 ± 6.7	93.3 ± 14.7	5.78
None.....	46.9 ± 2.5	58.3 ± 8.1	111.3 ± 16.0	8.80
Control Values.....	1081.2 ± 14.1 mm ³ - CO ₂ /hr/100 mg. Brain	1175.2 ± 41.6 mm ³ CO ₂ /hr/1 cc. packed RBC	511.3 ± 29.4 mm ³ CO ₂ 1 hr/1 cc. plas- ma	

¹ Standard error on % of mean.

TABLE 2. SIGNIFICANCE OF DIFFERENCE BETWEEN MEANS OF CHOLINESTERASE VALUES CORRESPONDING TO CATEGORIES OF TOXIC SIGNS FOLLOWING 2.0 MG/KG DFP
p Values determined in *t* test

SOURCE OF CHOLINESTERASE	SEVERE AND MODERATE	MODERATE AND SLIGHT	SLIGHT AND NONE
Brain.....	<.01 sig.	<.01 sig.	<.01 sig.
RBC.....	.02 ? sig.	<.01 sig.	.035 ? sig.
Plasma.....	<.01 sig.	.025 ? sig.	.24 not sig.

A value less than .01 means that the difference in the means is significant since this difference could only occur 1 time out of 100 by chance. Values from .01 to .05 are of questionable significance. Values greater than .05 indicate that the two means in question are not significantly different.

of the RBC cholinesterase values the relationship with toxic manifestations is not as clear-cut. The scatter is greater and each category is not as sharply demarcated from the next (table 2). The plasma cholinesterase values are even less consistent. The scatter is greater and the categories are not as well differentiated.

In a similar fashion table 3 indicates the relationship between the severity of clinical signs and the levels of the various cholinesterases in the rats injected with 1.0 mg/kg. Again the most consistent relationship is between the brain cholinesterase and toxic signs. Each clinical category is sharply demarcated and significantly different from the next as determined by the *t* test (table 4). As with the

larger dose, the relationship between clinical signs of toxicity and RBC cholinesterase is less consistent and plasma cholinesterase still less (tables 3 and 4).

It appeared of interest to compare the mean brain cholinesterase corresponding to categories of clinical signs following a 1.0 mg. and 2.0 mg/kg. dose with each other. This has been done in table 5. Furthermore, similar determinations previously made in the acute state (30 minutes following the injection of DFP) are also listed. A con-

TABLE 3. RELATIONSHIP OF SIGNS OF TOXICITY TO CHOLINESTERASE ACTIVITY FOLLOWING 1.0 MG/KG. DFP

SIGNS	BRAIN CHOL. % NORMAL	RBC CHOL. % NORMAL	PLASMA CHOL. % NORMAL	APPEARANCE OF SIGNS—MEAN TIME IN DAYS
Moderate.....	12.0 ± 1.1	19.5 ± 4.5	0	.042
Slight.....	31.6 ± 2.3	39.5 ± 7.1	77.8 ± 11.6	2.70
None.....	52.4 ± 2.9	68.4 ± 10.2	90.5 ± 11.8	7.43

Control values as in table 1.

TABLE 4. SIGNIFICANCE OF DIFFERENCE OF MEANS CORRESPONDING TO CATEGORIES OF TOXIC SIGNS FOLLOWING 1.0 MG/KG. DFP
p Values determined in *t*-test

	MODERATE AND SLIGHT	SLIGHT AND NONE
Brain.....	<.01 sig.	<.01 sig.
RBC.....	.05 ? sig.	.02 ? sig.
Plasma.....	<.01 sig.	.28 not sig.

See table 2.

TABLE 5. CORRESPONDENCE OF MEAN BRAIN CHOLINESTERASE PER CENT NORMAL AND CATEGORIES OF CLINICAL SIGNS UNDER VARYING CONDITIONS

CLINICAL CATEGORIES	PERIOD OF REGEN. FOL- LOWING 1 MG/KG. DFP	PERIOD OF REGEN. FOL- LOWING 2 MG/KG. DFP	30 MIN. FOLLOWING VARIOUS DOSES DFP ¹
	%	%	%
Moderate.....	12.0	17.9	11
Slight.....	31.6	35.9	33
None.....	52.4	46.9	

¹ From Freedman and Himwich (2)

sistency is observed in the level of the brain cholinesterase activity for each of the three states with the possible exception of those values corresponding to moderate signs.

Since in the acute state the RBC cholinesterase seemed to parallel the brain cholinesterase closely, it was of interest to note if any such correlation existed in the overall regenerative period studied in these experiments. The correlation coefficient of RBC with brain cholinesterase following a 1.0 mg/kg. dose of DFP was determined and found to be .74. Thus, one could use RBC cholinesterase to approximate

changes in the brain during regeneration as performed here. This would be more accurate than determining plasma cholinesterase both in the acute or recovery phase.

DISCUSSION

It appears from these data that there is a reversal of rates of regeneration between red blood cell cholinesterase and brain cholinesterase. In spite of the fact that erythrocytes cholinesterase returns to normal sooner than the brain, yet during the earliest period the brain regenerates more rapidly than the red blood cells. Thus the problem posed by Grob *et al.* (4) concerned with the early differences of the clinical signs may be explained by the early lag in erythrocyte regeneration in contrast with the simultaneous rapid regeneration of brain cholinesterase.

Our rats though exhibiting great powers of recovery nevertheless retained signs of toxicity longer than the human subjects. Needless to say such low levels of cholinesterase activity as reached in the rat were not obtained in man. Furthermore, these data indicating rapid destruction of cholinesterase by DFP and its relatively slower regeneration point out the potentialities of this drug for accumulation on repeated administration.

Additional correlative evidence is furnished in this work that toxic signs in DFP poisoning closely parallel changes in brain cholinesterase activity during recovery from a dose of DFP. It is possible that some of the toxic signs observed are not directly related to the central nervous system and it may well be that if one were to determine the regenerative pattern of cholinesterase activity in the neuromyal junction a similar or closer parallelism might be found. The fact remains however that this correlation exists between toxic signs and biochemical changes. It further appears that for each general level of brain cholinesterase activity one can expect approximately the same overt manifestations of toxicity as observed whether the animal is in the acute or recovery phase and this obtains whether a dose of 2.0 or 1.0 mg/kg. is used. It is noteworthy that brain cholinesterase activity can be reduced to about half before the earliest overt sign of toxicity develop. Of course, between the levels of 50 to 100 per cent normal cholinesterase activity changes in brain function may be present that require finer methods than those employed in the present investigation for their revelation. In any case, it should be pointed out a significant excess of cholinesterase appears to be present beyond that which is needed for normal function. All this suggests that brain cholinesterase plays an important role in the normal animal in the maintenance of nervous activity and that changes in brain cholinesterase activity are accompanied by corresponding alterations in the function of the central nervous system.

SUMMARY

Following the injection of large doses of DFP nonspecific plasma cholinesterase regenerates more rapidly than the specific cholinesterases of the red blood corpuscles and the brain. A lag in regeneration of erythrocyte cholinesterase activity lasting from 24 to 48 hours occurs following the injection of DFP, a period during which brain cholinesterase regenerates rapidly. Subsequently, the rate of regeneration of erythrocyte cholinesterase becomes more rapid and surpasses that of the brain. A close

relation appears to exist between the severity of the toxic signs of DFP poisoning and the level of brain cholinesterase during the regeneration period. The relation between toxic signs and erythrocyte cholinesterase activity is less exact and fails altogether in the case of the plasma.

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FACTORS IN EXPLOSIVE DECOMPRESSION INJURY¹

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CONVENTIONAL studies on explosive decompression have involved the sudden alteration of the environment from that of the normal, ambient atmosphere to rather high vacua, in simulation of pressure-cabin failure. In such experiments, therefore, test animals are simultaneously subjected to: a) the rapid expansion of contained gases; b) anoxic anoxia; and c) aeroembolism (at sufficiently reduced pressures). Qualitative evaluation of the relative importance of these factors in the etiology of injury and death from rapid decompression has thus been difficult. Other methods of experimental procedure were therefore adopted in the hope of further elucidating this phase of the problem.

METHODS

In over 300 individual tests, rats were compressed within 30 seconds to pressures of from 2 to 30 atmospheres, maintained for various time periods (10 seconds to 30 minutes). Subsequent decompression to normal atmospheric pressure was rapid (average time, 0.62 seconds, as determined by high-speed motion pictures). Adequate ventilation of the chamber was maintained throughout, the flow of air being measured by means of a gas meter. In this manner an anoxic environment was eliminated from the conditions of the experiment, and the effects of gas expansion, only, could be observed. It is obviously quite possible that nitrogen narcosis may have been present in animals placed under the higher pressures, but recognizable symptoms of this condition were not detected. It is moreover true that pressure changes in this type of experiment were many times greater than in tests of the conventional sort, but it was hoped to obtain by the method described an evaluation of the effectiveness of intrapulmonary gas expansion as a lethal agent.

The effects of anoxia were observed by placing animals in an atmosphere of nitrogen for suitable intervals. All rats which succumbed were examined for the presence of gross lesions, hemorrhage and the presence of gas emboli. Those surviving were kept under observation for periods of from 1 to 4 weeks, at the end of which time several rats from each experimental group were killed for study. In none of the latter animals, however, was gross evidence of decompression injury present at the time of autopsy.

The results of all experiments involving decompression are given in table 1. As shown in figure 1, explosive decompression following 30-minute exposures to 2 to 6 atmospheres (gas-expansion equivalent, 18,000 to 42,000 ft.) was well tolerated by 59

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rats. No gross pathological lesions were later observed in these animals. Following decompression from 7 atmospheres, half of the animals succumbed, without discernible injury, and mortality rates were slightly increased following release from 8 and 9 atmospheres of pressure. Decompression from 10 atmospheres resulted in the death of 14 of 15 rats (93%) and all (20) animals succumbed on rapid release from 11 atmospheres.

TABLE 1. EFFECTS OF EXPLOSIVE DECOMPRESSION FROM HIGH BAROMETRIC PRESSURE TO THAT OF AMBIENT ATMOSPHERE

PRESSURE FROM WHICH DECOMPRESSED	APPROXIMATE GAS EXPANSION EQUIVALENT ON DECOMPRESSION, EXPRESSED AS ALTITUDE	TIME AT HIGH PRESSURE	NO. OF ANIMALS TESTED	TOTAL DEATHS		ANIMALS SHOWING PULMONARY LESIONS		ANIMALS SHOWING AEROEMBOLISM	
				No.	%	No.	%	No.	%
<i>atmos.</i>	<i>ft.</i>								
2	18,000	30 min.	23	0	0	0	0	0	0
3	27,000	30	10	0	0	0	0	0	0
4	34,000	30	8	0	0	0	0	0	0
5	38,000	30	8	0	0	0	0	0	0
6	42,000	30	10	0	0	0	0	0	0
7	45,000	30	10	5	50	0	0	0	0
8	48,000	30	10	7	70	1	10	3	30
9	51,000	30	19	11	58	3	27	11	100
10	53,000	30	15	14	93	3	21	14	100
10	53,000	1	21	0	0	0	0	0	0
11	55,000	30	20	20	100	3	15	20	100
12	59,000	20	6	6	100	4	66	6	100
15	61,000	1	10	0	0	0	0	0	0
15	61,000	4	6	0	0	0	0	0	0
15	61,000	5	6	4	66	2	50	4	100
16.6	64,000	1	10	0	0	0	0	0	0
20	68,000	30 sec.	6	0	0	0	0	0	0
20	68,000	1 min.	18	9	50	7	77	9	100
20	68,000	2	9	4	47	1	25	4	100
20	68,000	3	9	6	66	2	33	6	100
20	68,000	4	12	10	83	4	40	10	100
20	68,000	5	6	6	100	1	17	6	100
23	71,000	1	6	4	66	0	0	4	100
30	76,000	10 sec.	16	0	0	0	0	0	0
30	76,000	30	12	8	66	4	50	8	100
30	76,000	50	12	12	100	0	0	12	100

Thus, of 74 rats, subjected for the same time-interval to demonstrably 'lethal' decompressions (7 to 11 atmospheres' differential), 57 (77%) succumbed. However, in only 10 animals (18%) was evidence of pulmonary hemorrhage seen at autopsy. On the other hand, in 48 of the rats (82%) killed by the decompression, there was definite aeroembolism (gas bubbles in the right heart and large vessels). It would hence appear that in these experiments, in which the animals were never anoxic, aeroembolism constituted the major lethal factor, rather than the physical expansion of the intrapulmonary gases.

This was further indicated by the fact that no deaths resulted in a series of 21

animals maintained under 11 atmospheres of pressure for only 1 minute prior to decompression (table 1). Moreover, since the average respiratory rate of sleeping and quiescent (resting) rats in the colony was found to be 82 and 115 per minute respectively (average of 50 determinations), one might safely assume the rather complete filling of the lungs with compressed air, even within 1 minute, and hence that all animals would be subjected to much the same degree of intrapulmonary gas expansion.

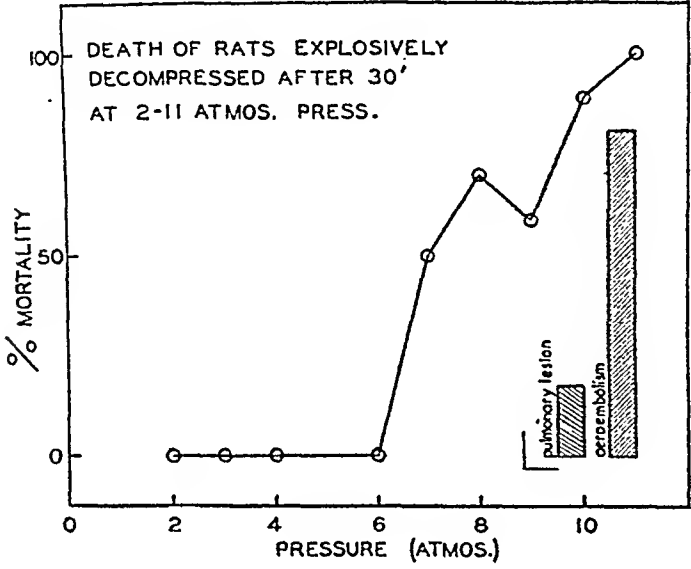
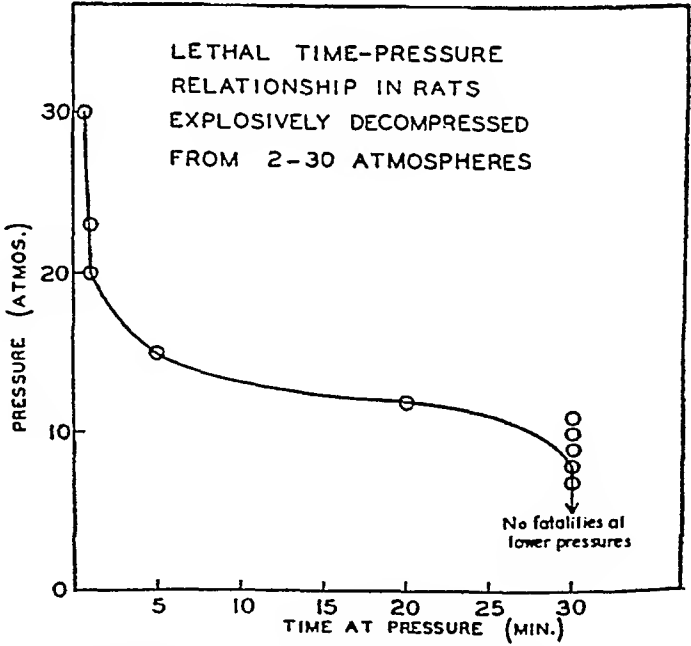


Fig. 1. MORTALITY RATE IN RATS explosively decompressed to ambient atmospheric conditions following exposure to 2-11 atmospheres of pressure.

Fig. 2. TIME-PRESSURE RELATIONSHIPS productive of lethal explosive decompression injury in the rat.

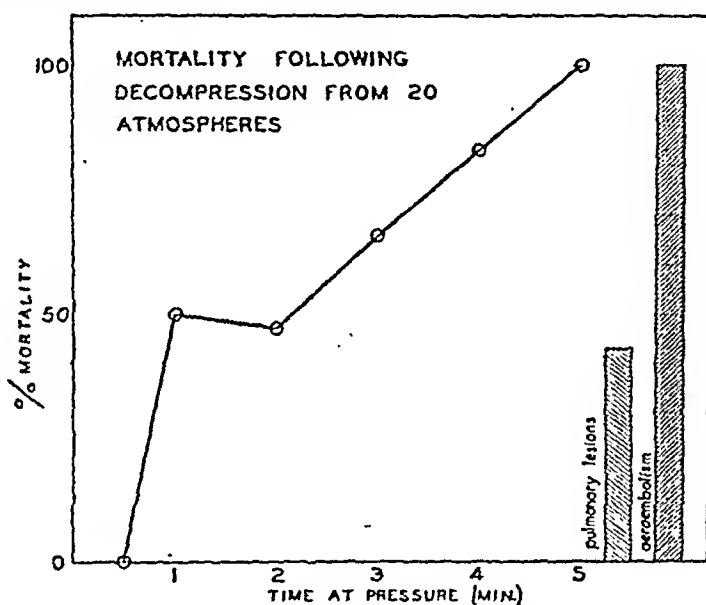


In the hope of finding a pressure differential at which gas expansion *per se* might invariably result in lethal trauma (as evidenced by pulmonary hemorrhage), experiments were continued to include decompressions from even higher pressures. Such tests gave increasing evidence that time under pressure (and hence aroembolism), rather than the expansion of intrapulmonary gases (fig.2) constituted, in these experiments, the major factor in survival. All animals died following decompression

from 12 atmospheres (gas-expansion equivalent, 57,000 ft.), and exposures to 15 atmospheres for only 5 minutes resulted in the death of 4 of 6 rats on depression. However, all survived when time under pressure was reduced to 4 minutes, and when this interval was further reduced to 1 minute 20 animals tolerated decompression from 15 to 16.6 atmospheres of pressure without fatality.

Further evidence that time under pressure constituted the major lethal factor in this type of experiment was obtained in a series of 60 rats decompressed from 20 atmospheres (gas expansion equivalent, 68,000 ft.). The results are shown graphically in figure 3. Of these animals decompressed after 30 seconds to 5 minutes under pressure, 35 died, with pulmonary lesions in only 15 cases (43%), while all rats which succumbed gave marked evidence of aeroembolism. Throughout the study 'pulmonary lesion' was recorded whenever the smallest ecchymoses could be grossly detected. Hence, in a large percentage of animals in which hemorrhage was noted, the amount of pulmonary bleeding could not have contributed significantly to the death

Fig. 3. MORTALITY RATE IN RATS explosively decompressed following exposure to 20 atmospheres of pressure for varying time intervals.



of the animal. Moreover, such pulmonary damage as was seen might, in many instances, have been due to the expansion of gas emboli within the smaller pulmonary vessels.

When decompressed after a 50-second exposure to 30 atmospheres all animals succumbed with marked aeroembolism. So great was the amount of gas within the heart that, on opening the thorax, an audible sound was, on occasion, produced by the cardiac contractions. All of the larger vessels exhibited emboli entirely blocking their lumina. However, no pulmonary bleeding was present in any of the 12 animals of this series. A reduction of the period of pressurization to 30 seconds resulted in the death of 8 to 12 rats, with pulmonary bleeding in 4 animals. All of the rats which succumbed showed marked aeroembolism. When time under pressure was further reduced to 10 seconds, all 16 rats survived. These results, like the foregoing, were interpreted as indicating that the physical expansion of the intrapulmonary air constituted a relatively minor contributing factor in causing death, and that in decompressions as here performed, i.e. in the absence of anoxia and following profound pressure changes, aeroembolism appeared to be the primary lethal agent.

ANOXIA

Twenty rats placed in an atmosphere of nitrogen succumbed after an average interval of 1 minute, 17 seconds, when respiration ceased. In half of these animals, pulmonary damage was observed at autopsy. Such lesions varied from small, discrete ecchymoses to diffuse hemorrhage involving all of the pulmonary lobes. It was therefore apparent that asphyxia alone may result in pulmonary bleeding in the rat, indistinguishable grossly from that seen following explosive decompression, probably as a result of the extreme and convulsive dyspnea induced. It may be noted that asphyxia does not invariably produce such lesions, nor does explosive decompression, whether performed in the conventional manner (1) or as in the present study.

DISCUSSION

It is of historical interest to note that Bert (2), in 1878, from a long series of studies on decompression injury concluded, "The physical phenomena amount to very little, even when the rapidity of the experiment should have increased their importance". Further, that 'the pulmonary ecchymoses' on release from pressure "mean nothing, because we find them in simple asphyxia, at normal pressure".

The pathology of explosive decompression injury is, in the main, at present quite well known (1, 3-8) and major differences of opinion among investigators appear to center about its chief etiological factors (6, 8-11). The determination of the principal causative agent or agents is of primary concern, since the design of any devices for the protection of aircrews from this hazard must depend on a knowledge of the mechanics and cause of injury.

Edelman *et al.* (3) are of the opinion that an increased intrapulmonary pressure is the primary etiological agent, and Whitehorn, Lein and Edelman have expressed a somewhat similar opinion (8). They considered aeroembolism to be a negligible hazard, following experiments with guinea pigs. Gelfan (9), on the other hand, considers anoxic anoxia to be the primary lethal agent. There is general agreement that rapid decompressions to simulated altitudes as great as 35,000 feet are entirely innocuous (1, 9).

It is interesting to note that lethal, simulated altitudes for the rat agree quite closely both in conventional studies and the present experiments. Thus Gelfan (9) found that rats succumb to anoxic anoxia at 52,000 feet (simulated), or higher while in the experiments described, a 93 per cent mortality occurred at a gas-expansion equivalent of 53,000 feet, and all animals died at the 55,000-foot equivalent on release from a 30-minute stay under pressure.

There is apparently little difference of opinion as to the value of rapid recompression (1, 10) to survival. This would obviously hold true whether the principal lethal factor be anoxic anoxia or aeroembolism, since both occur at sufficiently reduced pressures, and either condition may be corrected by recompression.

In experiments of conventional design, it is obvious that because of the comparatively small pressure differentials involved, aeroembolism would be of much rarer occurrence than in the experiments described. Quite recently, Hitchcock *et al.* (12), in experiments on human subjects found that when pressure oxygen breathing was employed, explosive decompressions to simulated altitudes up to 45,000 feet were well tolerated, without any evidence of bubble formation attributable to the explosive

decompression *per se*, although incapacitating bends and chokes did occur at extreme altitudes.

It would therefore appear that anoxic anoxia and aeroembolism constitute the major factors in causing death from explosive decompression, and that the physical expansion of intrapulmonary gases plays a relatively minor part in the etiology of injuries so produced.

SUMMARY

In experiments designed to eliminate the presence of anoxic anoxia, rats were explosively decompressed to normal atmospheric pressure after exposure to positive pressures of from 2 to 30 atmospheres for varying time intervals. Decompression from 2 to as much as 6 atmospheres (equivalent gas-expansion differential, 18,000 to 42,000 ft.) was well tolerated, and such decompressions may be considered to be entirely innocuous in the rat.

Of rats decompressed after 30-minute exposures to 7 to 11 atmospheres of pressure, 77 per cent died. Aeroembolism was present in 82 per cent of these animals, with pulmonary lesions in 18 per cent of cases. All animals succumbed following decompression from 11 atmospheres (gas expansion equivalent, 55,000 feet) when so maintained for 30 minutes prior to release of pressure. Without exception these animals exhibited gas emboli, while pulmonary hemorrhage was present in only 15 per cent.

All rats which died following explosive decompression from 20 atmospheres of pressure gave evidence of severe aeroembolism, while pulmonary lesions could be demonstrated in only 43 per cent of such animals. Decompression after a 50-second exposure to 30 atmospheres of pressure was invariably fatal. On the other hand, all survived this pressure change when time under pressure was reduced to 10 seconds. Pulmonary hemorrhage occurred in 50 per cent of rats dying from anoxic anoxia (nitrogen at normal atmospheric pressure).

These experiments indicate that anoxic anoxia and aeroembolism constitute the major factors in the etiology of explosive decompression injury, and that intrapulmonary gas expansion may be considered of relatively minor importance as a lethal agent.

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MINIMAL EFFECTIVE DOSE OF INTRAVENOUSLY ADMINISTERED HISTAMINE IN PREGNANT AND NON-PREGNANT HUMAN BEINGS

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IN VIEW of the problems which surround the question of the physiological rôle of histamine, this study was undertaken to determine the minimal effective dose of histamine administered by rapid intravenous injection. Reported data are scattered and incomplete, although the minimal effective dose of histamine given by continuous intravenous infusion in man has been carefully worked out for gastric secretion (1), facial flush and changes in cerebral blood vessels (2).

In view of the rise of the histaminolytic property of the blood which has been reported during pregnancy (3), it seemed desirable to determine the minimal effective dose in a group of pregnant females as well, in an effort to find evidence for a physiological rôle for this elevated blood 'histaminase'.

Harmer and Harris (4) gave 20 micrograms of histamine base¹ intravenously on 12 occasions to an unspecified number of patients, all of whom experienced metallic taste and facial flush. Almost always they felt warm, and frequently, although less constantly, they experienced throbbing in the head. Best and McHenry (5) noted that 15 micrograms of histamine gave a metallic taste, tightness of the head and headache. Pickering and Hess (6) produced headache regularly with 40 micrograms of histamine. Weiss and co-workers (2) stated that the minimal intravenous dose of histamine that would exert changes in the cerebral and facial vessels was 7 micrograms of histamine, as measured by flush and rise in cerebro spinal fluid pressure.

Storch (7) chose 20 micrograms as the "active subthreshold dose" because he found that it did not produce headache in most instances in normal individuals.

Rothlin and Gundlach (8) obtained no gastric response in dogs by the rapid intravenous injection, in six instances, of doses of histamine varying from 0.05 mg to 0.4 mg.

METHODS

This study employed 36 white adult subjects: 12 normal males, 14 non-pregnant females, and 10 pregnant females in the second and third trimesters of pregnancy.

Histamine dihydrochloride, diluted with physiologic saline so that 1 ml. of solution contained 20 micrograms of histamine base, was administered intravenously as rapidly as possible through a 23-gauge needle in volumes which ranged from 0.2 ml to 1.5 ml. The subjects were seated comfortably in a good light. To avoid the distracting influence of the venipuncture the intravenous injection was made a short time after the needle was introduced into the vein following the release of the tourniquet. The subjects were unaware for the most part of the expected reactions, and

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¹ All doses of histamine are calculated as histamine base.

were simply asked to describe in detail all their sensations as they occurred, while they were being observed for facial flush.

The rapid intravenous injection of histamine produced the responses which have been described in detail by others. A metallic taste was perceived usually 20 to 30 seconds after injection, followed by a marked facial and neck flush (25-35 seconds after injection). This was usually accompanied by sensations of warmth over the entire face and forehead. Between 40 and 60 seconds after injection, dizziness, lightheadedness and vertigo were felt, associated with the sensations of pressure, throbbing and ache or pain within the head. The headache or pain produced by the doses employed reached a peak of intensity within 1 to 1½ minutes, and subsided promptly within 2 to 3 minutes after injection.

After some preliminary observations the initial dose employed was usually 4 or 10 micrograms. These were randomized and repetition of intravenous doses was done no sooner than at 15-minute intervals, or after all symptoms had disappeared. In 30 subjects only two different doses were required to elicit clear-cut differences of response; in 6 subjects three different doses were required, although threshold doses for every type of response were not obtained in every individual, in the range of doses tested.

Five control subjects were retested one week following the original observation. There was complete reproduction of their original responses at the same dose levels.

RESULTS

From the results summarized in table 1 and figure 1, it is apparent that for any given individual the minimal effective dose of histamine is not the same for all of the phenomena studied. It is apparent also that doses of histamine of the range of 2 to 4 micrograms are capable of stimulating taste receptors and effecting changes in facial and cerebral blood vessels in 25 per cent of the subjects studied, and that doses of 10 micrograms effected these changes in 75 per cent of the subjects.

Further, the range of minimal effective doses of histamine in the group of pregnant subjects is within the range of the minimal effective doses of the control group.

DISCUSSION

The changes induced by histamine in this study fall into three groups: 1) changes in taste receptors, 2) changes in facial vessels manifested by flush and sensations of warmth and 3) accepting Pickering's analysis (6), changes in cerebral blood vessels manifested by sensation of dizziness, and headache or pain.

Although our values are somewhat smaller, our findings are in keeping with those reported by Weiss *et al.* (2), who studied cerebral vascular changes by the rise of cerebrospinal fluid pressure. They clearly indicate the extreme sensitivity of these vessels. With headache and flush as our criteria we were unable to note any marked difference between cerebral and facial blood vessels.

It is of interest in this connection that the minimal effective dose of histamine administered by continuous infusion in man is 0.004 $\mu\text{g/kg/min.}$ for gastric secretion; 0.001-0.04 $\mu\text{g/kg/min.}$ for facial flush, and 0.02-0.05 $\mu\text{g/kg/min.}$ for headache (1, 2).

The finding that the minimal effective dose of histamine is the same for the

pregnant group as for the control group is of considerable interest in view of the marked rise in histaminolytic power of the blood in pregnancy. Ahlmark (3) in a

TABLE 1. MINIMAL EFFECTIVE DOSE OF HISTAMINE IN MICROGRAMS OF BASE

SUBJECT	SEX	TASTE	FLUSH	WARMTH	DIZZINESS OR VERTIGO	HEAD PAIN OR HEADACHE
1	M	10	10	20	20	20
2 ¹	M	10	10	10	20	20
3 ¹	M	4	4	10	10	10
4 ¹	M	10	10	10	10	10
5	M					10
6	M		4	10	10	4
7	M		10		4	
8	M		4	10	4	10
9	M	4	10	4		4
10	M	10	10	10	20	4
11	M	10	10	10	10	10
12	M	10	15			10
13 ¹	F	2	4	2	2	2
14	F	10	10	10	10	10
15	F		10	4		10
16	F	4	10	10	10	10
17	F	10	10	4	4	4
18	F	4	4	4	4	20
19	F	20	10	10	10	20
20	F	10	10	4	10	10
21	F	30	20	10	20	30
22	F	10	10			10
23	F	10	4	4		10
24	F	4	10	10	4	4
25	F	4	4	10	10	4
26	F	10	4	4		10
	MONTH OF PREGNANCY	TASTE	FLUSH	WARMTH	DIZZINESS OR VERTIGO	HEAD PAIN OR HEADACHE
27	9	10		10		10
28	8	10		4	4	
29	7	4	10	10	4	4
30	8	10	10	10	10	10
31	8	4	10	10	10	10
32	7	10	10	10	10	15
33	6	4	4	4	10	10
34	6	4	10	10	10	10
35	6	15	15	15	10	15
36	5	10	10	10	10	4

¹ Retested subjects.

recent and careful study has reported that during the second and third trimesters, on the average, 1 ml. of plasma of a pregnant woman will inactivate 5 micrograms of histamine per hour. This is equivalent to 0.2 Winthrop unit of histaminase per ml. of blood.

It has recently been shown in the dog that the intravenous injection of 20 units of hog kidney histaminase per kilogram of body weight, which is equivalent to 0.4 units per ml. of blood and which is capable of inhibiting the minimal effective gastric secretory dose of histamine, does not inhibit the vasodepressor response of a minimal effective dose of histamine given intravenously (9). This minimal effective vasodepressor dose is six times larger than the minimal effective dose for gastric secretion (1). However, larger doses of histaminase (1000 U/gm.) definitely inhibited the vasodepressor action of histamine in cats (9).

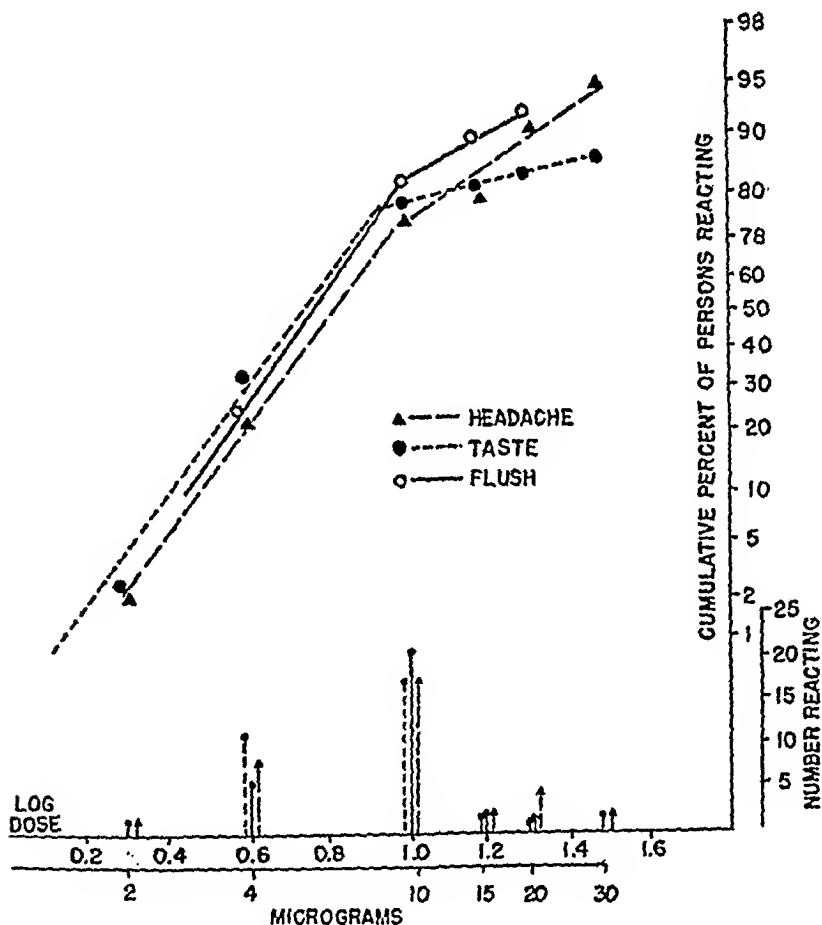


Fig. 1. VERTICAL LINES SHOW FREQUENCY DISTRIBUTION of the number of reactors at each dose level. Sloping lines show the cumulative percentage of persons reacting at the various dose levels. The percentage scale is in probability units so that a straight line would result if there were a normal frequency distribution. The fact that the cumulative percentage lines are truncated indicates an anormal frequency distribution.

It should be emphasized that the speed with which an enzyme acts depends, among other factors, upon the concentration of the enzyme. The failure of the histaminase of pregnancy to elevate the threshold for the vascular responses to intravenously injected histamine reflects an inadequate concentration rather than slowness of reaction. That such enzymatic actions may occur within a matter of milliseconds in the presence of adequate concentrations of enzyme has been shown by Nachmansohn for the cholinesterase of neural tissue (10). It is also possible that the blood 'histaminase' of pregnancy may be capable of neutralizing the minimal effective

gastric secretory dose, although by the method of rapid intravenous injection of histamine no elevation of threshold was elicited in the pregnant group.

SUMMARY

Two to four micrograms of histamine base injected rapidly intravenously stimulated taste receptors and effected changes in facial and cerebral blood vessels in 25 per cent of the subjects tested. Ten micrograms of histamine effected these changes in 75 per cent of subjects tested. The range of minimal effective doses of histamine given rapidly intravenously is the same for pregnant and non-pregnant subjects.

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WORK PERFORMANCE OF NORMAL RATS GIVEN CONTINUOUS INJECTIONS OF ADRENAL CORTEX EXTRACTS

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IT HAS been established that the resistance of non-adrenalectomized animals to certain types of stress can be increased by the administration of adrenal cortex extracts and steroids. However, the known conditions where these hormones benefit non-adrenalectomized animals are few and the results of most studies of this general problem have been negative. The field has not been fully explored and some claims of positive results will require confirmation before they become acceptable.

In the early years following the isolation of potent adrenal cortex extracts there were a number of reports that normal animals were made more resistant to fatigue by the administration of adrenal cortex extracts. The reports (1-3) of a synergistic effect of adrenal cortex extract and ascorbic acid upon the work performance of non-adrenalectomized rats prompted one of us (D. J. I.) to carry out an extensive series of studies (unpublished) of this problem during 1936 to 1938. Adrenal cortex extracts and ascorbic acid were tested separately and in combination upon the ability of normal rats to work in the following situations: 1) the swimming time of the unanesthetized rat weighted with 10 gm. and weighted with 20 gm.; 2) the running time of the unanesthetized rat in a motor-driven revolving cage; 3) the work of the gastrocnemius muscle of the anesthetized rat during 120 hours of faradic stimulation. Amounts of adrenal cortex extracts up to 3 cc. per day (75 gm. of gland per cc.) were tested in each study. All of the results were negative.

More recently we have developed an apparatus for the continuous injection of hormones and a modification of the muscle-work test (4) which permits the activation of all of the musculature of both back limbs. This procedure is a severe stress which rapidly produces hypoglycemia in normal rats unless glucose is administered. Some working normal animals develop hypoglycemic convulsions and die within 24 hours. Since adrenal cortex extract tends to stimulate gluconeogenesis and to conserve carbohydrate in both adrenally insufficient and normal animals, at least under resting conditions, and since the cortical hormone requirement of the adrenally insufficient rat is very high under these conditions (5) we have reinvestigated the effect of continuous injections of adrenal cortex extract upon the work performance and survival of the non-adrenalectomized rat. The results were negative.

METHODS

Male rats of the Sprague-Dawley strain which weighed 200 ± 2 gm. were used. These animals were free from parasites and infections. The diet was Archer Dog

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Pellets. The procedures used for the stimulation of muscle were according to Ingle (4) with the following modifications. A Nerve Stimulator, Model B, Upjohn, was used to stimulate muscle at the rate of 5 times per second. The duration of each pulse was 20 milliseconds and the intensity was 20 milliamperes. An electrode was placed on the lower tibia of the left back leg and the second electrode on the contralateral back foot, thereby activating all of the musculature of both hind legs. The gastrocnemius muscle of the left hind leg was weighted with 100 gm. The distance that the weight was lifted was registered on automatic work recorders. Each recorder revolution represented approximately 400 gm.-cm. of work.

A solution of 0.9 per cent sodium chloride with and without beef adrenal extract was either given by subcutaneous injection or it was infused into the jugular vein at a constant rate by means of a constant injection apparatus which simultaneously delivered fluid from each of 12 syringes at the rate of 20 cc. in 24 hours. The adrenal cortex extract represented 40 gm. of beef adrenal extract per cc. and was free from alcohol. All doses of ACE were diluted to 20 cc. with saline. Temperature was constant at $28 \pm 0.5^{\circ}\text{C}$.

The animals were anesthetized with phenobarbital sodium and cyclopal sodium (4). They were subjected to the work tests as soon as surgical anesthesia was attained. Stimulation was continued until the muscle ceased to respond or for 24 hours.

EXPERIMENTS AND RESULTS

The data of these experiments are summarized in table 1. Twelve pairs of rats were represented in each experimental group. The animals treated with ACE and their controls were always tested simultaneously.

Three groups of rats were tested without prior fasting. The doses of ACE were 5, 10 and 20 cc./rat/24 hours. The solutions were given by subcutaneous injection.

Two groups of rats were fasted for 24 hours prior to the beginning of the work test. The amounts of ACE were 10 and 20 cc./rat/24 hours. The solutions were given by subcutaneous injection.

One group of rats was fasted for 24 hours prior to the beginning of the work test. One rat of each pair received 20 cc. of ACE per 24 hours by intravenous injection.

Two groups of rats were fasted 32 hours prior to the beginning of the work test. One rat of each pair was given 2 cc. of ACE by subcutaneous injection at 8, 6, 4 and 2 hours prior to the beginning of the work test. The control animals received saline injections. The experimental animals received 20 cc. of ACE/rat/24 hours during the work test. The results of these two groups are combined together in table 1.

There was no significant difference between the average amounts of work performed by rats which received ACE and their controls which received saline in any of the experimental groups. The average amount of work done by each of the 96 rats which received ACE was 19,470 recorder revolutions and the average for all of the control animals was 19,465.

There was no tendency for the administration of ACE to reduce the number of deaths from hypoglycemia during work. Among the 36 pairs of non-fasted rats 4 of the ACE rats died and 6 of the saline controls died. Among the 36 pairs of rats

which fasted 24 hours, 11 of the ACE rats died and 10 of the saline controls died. Among the 24 pairs of rats which fasted for 32 hours, 8 of the ACE (pre-treated) rats died and 4 of the saline controls died.

One group of rats was fasted for 32 hours and one rat of each pair was given 2 cc. of ACE at 24, 26, 28 and 30 hours by subcutaneous injection. The control animals were given equal volumes of saline. At 32 hours the animals were anesthetized with cyclopal and the liver glycogen was determined according to Pabst *et al.* (6). The

TABLE 1. WORK PERFORMANCE OF NORMAL RATS GIVEN CONTINUOUS INJECTIONS OF ACE (BEEF ADRENAL CORTEX EXTRACT)

ACE PER 24 HRS.	ROUTE OF INJECTION	FAST PRIOR TO WORK, HRS.	PRE-TREAT. PRIOR TO WORK, HRS.	NUMBER PAIRS OF RATS	TOTAL WORK ¹ , AVERAGES AND RANGE	
					ACE	Control
cc.						
5	SQ	0	0	12	24940 15472-31014	22132 5109-37875
10	SQ	0	0	12	25934 10478-40143	23865 5937-37333
20	SQ	0	0	12	23519 10869-33353	24780 10824-36001
10	SQ	24	0	12	12506 2179-23271	15259 4420-25398
20	SQ	24	0	12	16485 5711-31720	14124 7461-24670
20	IV	24	0	12	16745 8142-25450	18904 9996-24808
20	IV	32	8	24	17816 5236-26490	18329 7285-25420

¹ Work is expressed as recorder revolutions. Each recorder revolution represents approximately 400 gram-centimeters of work.

Average for all ACE, 19,470; for all controls, 19,465.

average amount of glycogen in the livers of the ACE rats was 56.7 mg. and the average amount in the livers of the controls was 5.33 mg.

DISCUSSION

We have been unable to show that the work performance of the non-adrenalectomized rat is improved by the administration of large amounts of adrenal cortex extract. The secretory activity of the adrenal cortices of the intact rat is apparently capable of meeting the needs of the organism under these conditions. However, it has been shown that the removal of one intact adrenal does limit the ability of the rat to work (5).

Since the normal rat develops hypoglycemia and may die under the stress of work, it was anticipated that the administration of ACE should offer some protection against collapse from hypoglycemia just as the resistance of the normal rat (7) to insulin can be raised by the cortical hormones. This did not occur even when the rats were fasted prior to work. Pretreatment with ACE did increase the level of liver glycogen ten-fold but the actual energy value of this increase in liver glycogen is negligible when the carbohydrate requirement of the working rat is considered (8).

SUMMARY

Normal rats were subjected to the faradic stimulation of both hind legs at a rate of 5 times per second until death occurred or for a period of 24 hours. The continuous subcutaneous and intravenous administration of adrenal cortex extract in amounts of 5, 10 and 20 cc. of beef adrenal extract/rat/24 hours failed to improve the work performance of either fasted or non-fasted rats. The pretreatment of the fasted rat for 8 hours prior to the beginning of the work test failed to either improve the work performance of the animals or to protect against collapse (hypoglycemic), although this procedure caused a ten-fold increase in liver glycogen in a similar series of animals.

We wish to express our appreciation to Miss Joan E. Wilhelm who carried out the determinations of liver glycogen in this study.

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ACUTE HYPOTHERMIA IN GUINEA PIGS¹

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MOST investigators of hypothermia in mammals agree that anoxia plays a significant rôle, if not the dominant one, in death by cold. Although the literature contains no conclusive proof that the diminution in oxygen consumption during hypothermia is relatively greater than the concomitant reduction in the oxygen requirement, much evidence supports this thesis. The major differences of opinion among investigators of this problem arise in defining the mechanism or mechanisms by which anoxia occurs.

Pioneer experimenters were impressed with the profound depression of respiration, leading eventually to apnea often before cardiac standstill. Their observations have led to the conclusion that hypothermia is responsible for a direct inhibition of the respiratory reflexes and that the ensuing anoxia is predominantly anoxic in type. In this regard the apparently beneficial influence of artificial respiration is often cited. On the other hand, Crismon (1) believes that a stagnant anoxia is the critical factor, arising from a suppression of cardiac output as evidenced by a profound slowing of heart rate and terminal drop in arterial blood pressure; in his opinion apnea is only secondary to cardiac failure. German experiments summarized by Alexander (2) support a similar thesis. Werz (3) has furnished evidence that a lowered rate of dissociation of oxyhemoglobin may be a limiting factor in the availability of oxygen for tissue metabolism. Dill and Forbes (4) have calculated that in spite of presumably high alveolar O₂ pressures the arterial oxygen tension was significantly reduced by cooling their human subjects; they suggest that slow diffusion of oxygen across the alveolar walls gives rise to anoxia. Denying the crucial rôle of anoxia, Grosse-Brockhoff and Schoedel (5) stress a direct hypothermic paralysis of medullary centers and an impairment of processes of excitation and conduction in the heart.

The data reported here represent diverse physiological observations on unanesthetized guinea pigs during and following cooling in an air jacket surrounded by ice water. These measurements include responses of colonic temperature, heart rate, ventilation rate (minute respiratory volume), oxygen consumption and carbon dioxide output. Attempts are made to correlate these physiologic indices with the observed behavior of each animal and with its success or failure to survive these acute and severe reductions of body temperature.

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PROCEDURE

Guinea pigs of both sexes ranged in body weight from 250 to 380 gm. Each experimental animal was carefully restrained by adhesive tapes attached to a wire mesh which formed an open cylinder loosely encircling the torso. The animal and its restraints were then inserted into a large surgical rubber glove which was suspended vertically and lowered into an ice water bath so that only the open end of the glove and the animal's head remained above the water surface. By this technique the animal's fur remained dry throughout (except on the perineum due to urination). Because an appreciable but variable amount of air was trapped in the glove during the immersions, rates of cooling were slower and presumably more variable than those that might be observed with direct immersion; perhaps the dry fur lent more uniformity to subsequent rates of rewarming in air. After a severe reduction in body temperature (to 17–21°C.), the water bath and glove were removed, and the animal was allowed to rewarm in still air (usually at either 25° or 27.5°C.). Although many animals were maintained in a vertical posture for 6 to 8 hours, no significant changes in rectal temperature, heart rate, ventilation rate or oxygen consumption could be detected when the animal was restored to a prone position before or after the immersion.

An index of the temperature of deep tissues was obtained with a calibrated constantan-iron thermocouple inserted per rectum so that the junction lay within the colon at a depth of 6 to 8 cm. (approximately at the level of the hilus of the left kidney). When the junction at this depth was moved 1 or 2 cm. in either direction, the recorded temperature did not change by more than $\pm 0.1^\circ\text{C}$. Although the junction was exposed, both insulated wires from it were encased in a soft rubber catheter, and the entire unit was firmly held in place throughout the entire period of cooling and rewarming. A recording potentiometer automatically registered the thermoelectric voltage every 3 minutes throughout the test. The sensitivity of the apparatus was $\pm 0.1^\circ\text{C}$.

In 9 tests, needle electrodes connected to a standard string galvanometer were inserted subcutaneously over the precordium and in the groin. Electrocardiograms were photographed periodically during both cooling and recovery. Heart rates measured from these records were further supplemented at low rates by counting directly the oscillations of the galvanometer string.

The measurement of ventilation rate (minute respiratory volume) in small unanesthetized mammals offers several technical problems. A respiratory mask was eventually constructed which proved air-tight on all guinea pigs tested. The crucial feature of the mask was a broad tight cuff of dental dam acting both as an air seal around the neck and as a harness to hold the mask in place. Around this cuff was wrapped a long strip of dental dam about 5 cm. wide; it extended well over the animal's shoulders. Small rubber flap valves connected to the mask offered negligible resistance to both inspiration and expiration. Generally room air entered the inspiratory valve but occasionally pure oxygen was introduced. Expired air was collected in a small spirometer (capacity 1 liter) and excursions of the spirometer drum were recorded continuously by a pen on a moving paper belt. As calculated from the slopes of these lines, our values of ventilation rate are dependable within 5 to 10 ml/min. Each recorded value of the minute respiratory volume represents the average response of several minutes (generally 2 minutes but as long as 10 minutes during the respiratory depression of severe hypothermia). Volumes of expired air were measured at 25°C. and, in general, ventilation rates have not been corrected from that to body temperature.

Ventilation rates measured prior to cooling appeared inappropriately high, even after acknowledging that these unanesthetized animals were not in a truly resting state as judged by their intermittent but persistent struggling. Thus initial values in various experiments ranged from 360 to 520 ml/min. (average 420). Although the mask and valves were designed so that the dead space was only a few ml., any dead space in the apparatus is made significant by the inevitably rapid breathing and low tidal volume of these small mammals. Although this respiratory dead space must have varied from test to test it remained relatively constant in any one experiment. Therefore, relative changes in the observed ventilation rate during the course of hypothermia are valid, even though absolute values are of doubtful physiological significance. For this reason, all ventilation rates are here reported in percentage of the initial rate prior to immersion, a value obtained by averaging several observations over a period of at least one hour. These duplicate determinations seldom varied from their mean by more than 5 per cent.

In 6 tests, samples of expired air were taken periodically from the spirometer and analyzed for

oxygen and carbon dioxide. The gas analyzer, described by Rahn *et al.* (6), employs a Pauling oxygen tensimeter and a Cambridge thermoconductivity meter for carbon dioxide. Because of dead space within the mask and particularly because of a common pathway from the mask to inspiratory and expiratory valves, samples of expired air were relatively high in oxygen (e.g., 18.7%) and low in carbon dioxide (e.g., 1.9%). It is apparent, however, that absolute values of oxygen consumption and carbon dioxide production calculated from these analyses are not invalidated by such dead space. Values reported are expressed in mm³/min/gm. of body weight, and they are undoubtedly dependable within ± 10 per cent.

RESULTS

Behavior. The average pre-immersion colonic temperature was 38.1°C. (standard deviation ± 0.75). Within 3 to 6 minutes of exposure to cold, the body temperature invariably fell; between 35 and 22°C., the rate of cooling was sensibly linear (0.2 to 0.3°C. per min). Shortly after the immersion, all animals intensified their struggling. Respiratory and ventilation rates accelerated during this initial period. Some animals consistently vocalized, others never. Gnawing was a constant feature of their efforts to escape. Tearing, salivating and chewing were prominent on occasion. Shivering as such was not invariably present, the increased motor activity often being of a jerky convulsive character. Such purposeful activity generally ceased at about 26°C., shortly after the beginning of a gradual fall in ventilation. When present, shivering disappeared a few degrees lower; it was often superseded by a slow rhythmic tensing of the limbs. By 20°C., all extraneous motor activity had generally disappeared; muscles became flaccid; respirations were slow, shallow and gasping (though seldom irregular). There were no detectable responses to cutaneous stimulation.

A critical but sublethal exposure was intended, but no single criterion proved to be a reliable signal for terminating the cooling. As judged in these tests, the colonic temperature itself was not crucial. Some animals survived briefly at 17°, others succumbed at temperatures between 20° and 21°. As demonstrated later, data on heart rates and ventilation rates are valuable but not predictive, since even apnea and ventricular fibrillation may be reversible. The decision to terminate an immersion remained arbitrary. Minimal temperatures ranged from 17.1 to 21.8°, averaging 19.3° C. When the guinea pigs were restored to still air at 25°C. (or 27.5°), colonic temperatures continued to fall for 9 to 12 minutes and then rose slowly.

At this point, heat production was generally so low that the living animal warmed little faster than the dead one. Gradually heat production assumed the major rôle in repaying the thermal debt. Relative to the motor activity during cooling, rewarming was a peaceful operation. A few animals were restored to normal colonic temperatures without displaying any detectable shivering or struggling. Generally, however, both appeared, though only at higher temperatures than those at which they had ceased during cooling. However intense this activity during the recovery process, it generally lessened after 3 to 4 hours. Simultaneously, the ventilation rate and oxygen consumption fell. As a result the colonic temperature tended to level off, often far short of normal levels.

Heart Rates and Electrocardiograms. Figure 1 illustrates the progressive fall in heart rate which accompanied cooling (9 tests). It is noteworthy that cardiac slow-

ing appeared as soon as there was a detectable fall in colonic temperature, in spite of the frenzied physical exertion which characterized the early stages of cooling. To be sure, the rate of fall was slightly slower during the initial decrement of 2 to 3° C. than thereafter. Between 36 and 23°C., the relationship between colonic temperature and heart rate was sensibly linear. The regression line (obtained by the method of least squares) had a formula $y = +17.5x - 312$ (where y = heart beats per min. and x = colonic temp. in °C.). Comparable data on adult anesthetized rats reveal a slope of 15 beats per minute per °C. (1); and on unanesthetized rats 19 beats per minute per °C. (7); but at all temperatures, rates in rats are 50 to 75 beats per minute higher than in guinea pigs. The latter difference may be a significant factor in the greater susceptibility of guinea pigs to a hypothermic death.

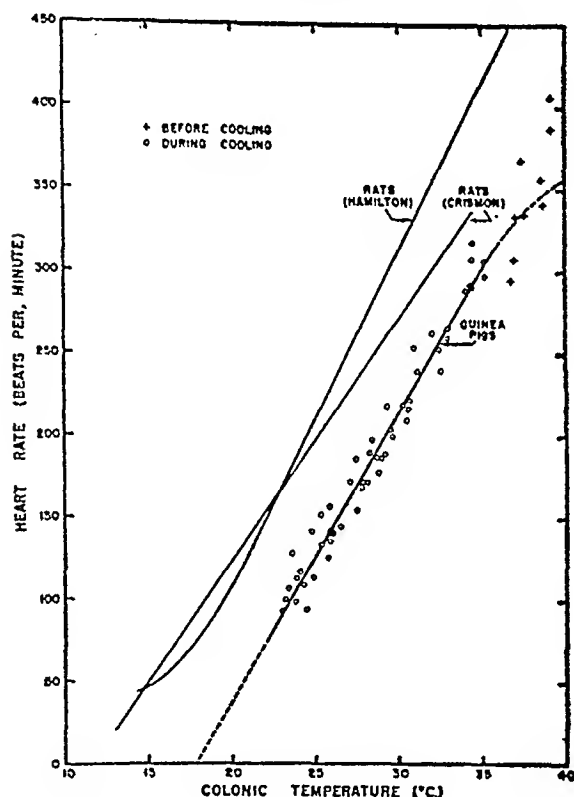


Fig. 1. RELATIONSHIP BETWEEN HEART RATE and colonic temperature in 9 guinea pigs during cooling between 35° and 23° C. Lines for unanesthetized rats after Hamilton (7), for anesthetized rats after Crismon (1).

The high correlation illustrated in figure 1 suggests that the body temperature is a direct determinant of heart rate. The relationship appears to be independent of the rate of cooling. When the rectal temperature was held at a constant low level (21.6°C., fig. 6), the heart rate remained relatively stable for several hours. Below 20 to 22°C., however, the heart rate became erratic. In some tests, the rate leveled off and in others it dropped precipitously. At rates below 70 beats per minute, the rhythm generally became irregular. These arrhythmias usually appeared to be of sinus or nodal origin. Once established, the arrhythmia generally persisted until a rise in body temperature had accelerated the rate to well over 100. In one test (fig. 5), an irregular ventricular rate of not over 13 beats per minute persisted for 30 minutes and included short episodes of ventricular fibrillation, after which a normal sinus rhythm returned as the animal warmed in air.

Because of muscle action potentials, no detailed electrocardiographic analysis was possible. Data on anesthetized rats have been well summarized by Crismon (1). In his studies abnormalities associated with cooling included prolongations of the PR, QRS and ST intervals, voltage increases, changes in the contour of the P- and T-waves, elevations of the ST segment, production of various degrees of A-V block, sinus and nodal arrhythmias, complete disappearance of P-waves, and ectopic ventricular beats. Many of these phenomena were identifiable in our records. A slow bizarre pattern diagnostic of ventricular fibrillation, seen once, has not been previously described in hypothermia. The mechanism of origin of these abnormalities has recently been investigated by such techniques as vagotomy (5), local heating of the heart (8), artificial respiration (1), and injections of atropine, glucose, calcium and cardiac glycosides (9, 10). Crismon (8) believes that terminal arterial hypotension in cooled rats is due to this bradycardia (a direct temperature action on the pace-makers and conducting mechanism) and to impaired ventricular emptying (an expression of inadequate myocardial nutrition). According to him, circulatory collapse is the critical feature of hypothermic death in rats. Data on guinea pigs are not complete enough to appraise the functional significance of the electrocardiographic findings.

All investigators agree that an animal which has survived a severe cooling carries no stigma of residual cardiac damage. In the present study, too, electrocardiograms returned to normal. In the early phases of recovery, arrhythmias disappeared as the rate rose. Although rewarming was 4 to 5 times slower than cooling, the correlation between heart rate and colonic temperature was essentially the same whether the temperature was rising or falling. A tendency was noted, however, for the rate at a given temperature to be slightly higher during the cooling. This may be due to the probability that heart temperature was slightly higher than colonic temperature during cooling and conversely during rewarming. If this interpretation is correct, the two temperatures never differed by more than 0.5°C .

Ventilation Rate and Oxygen Consumption. Figure 2 demonstrates the relative changes in ventilation rate during the cooling of 15 guinea pigs (dark line is the 'average'). Within 1 to 2 minutes of the immersion, before any drop in deep body temperature, struggling increased and hyperventilation began, presumably due to cutaneous stimulation. Maximal ventilation rates, however, did not appear until an appreciable reduction in colonic temperature (average 35°C .), and amounted to 30 to 100 per cent above pre-immersion values (average 70%). Below 35°C . ventilation rates declined steadily, reaching control values at 26° to 28°C . and approaching zero usually below 20°C . The variability shown in figure 2 is unexplained, but those animals with rates well below the 'average' line died in spite of their relatively mild exposures. Apnea, however, does not necessarily preclude a spontaneous recovery provided rewarming is commenced promptly after the cessation of breathing. For example, while in room air immediately after its immersion, one animal was apparently apneic for 20 minutes, during which time its colonic temperature rose 1.2°C . At this point, breathing reappeared spontaneously, and its recovery was subsequently uneventful.

The literature contains several detailed analyses of the relation between the oxygen consumption of guinea pigs (and other small mammals) and the envi-

ronmental air temperature (11), but a systematic comparison with the body temperature in deep hypothermia was not found. In the present study values of oxygen consumption (fig. 3) paralleled those of ventilation rate. The average initial rate of $20.8 \text{ mm}^3/\text{min}/\text{gm.}$ is 50 per cent higher than values reported for resting metabolism (11, 12), a difference readily ascribable to struggling even before the immersion. Maximal rates of 33 to $38 \text{ mm}^3/\text{min}/\text{gm.}$ (2-3 times estimated basal) occurred at colonic temperatures of 35 to 36°C. These values are 90 per cent above the peak metabolism observed by Herrington (11) when he exposed guinea pigs to cold air at 14°C. At temperatures below 27 to 29°C. , oxygen consumption dropped below pre-cooling rates and thereafter declined steadily along with the

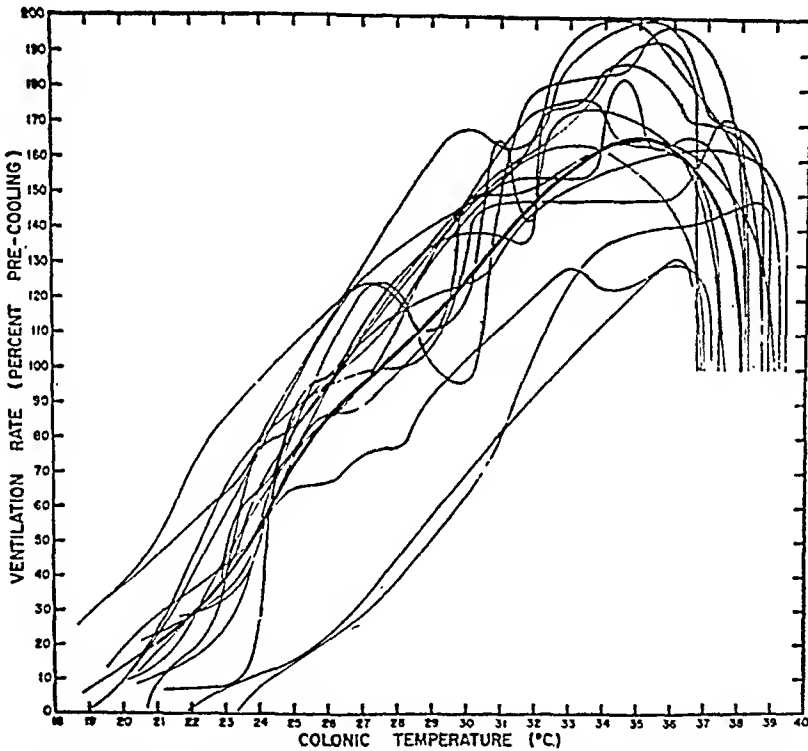


Fig. 2. RELATIONSHIP BETWEEN VENTILATION RATE and colonic temperature during cooling of 15 guinea pigs. Dark line is the line of 'averages' in the ordinate.

ventilation rate. With anesthetized dogs immersed in cold water, Grosse-Brockhoff and Schoedel (5) observed maximal rates of oxygen consumption at rectal temperatures of 32 – 33°C. ; these rates were 100 to 300 per cent above initial values. In men with rectal temperatures of 35°C. , Dill and Forbes (4) observed metabolic rates three times basal.

It is instructive to compare rates of ventilation and of oxygen consumption. The oxygen ventilatory equivalent (ventilation rate per 100 ml. oxygen uptake) is a convenient index for this purpose. In calculating this ratio, volumes of expired air (ventilation rate) were first converted to those at colonic temperatures (i.e., estimated lung temperature). Dill and Forbes (4) observed high ventilatory equivalents in human beings (schizophrenic patients) cooled to rectal temperature of about 30°C. , at which point oxygen consumptions were still above basal. Figure 4 demonstrates

that in guinea pigs this ratio remained high even during the severe respiratory and metabolic depression of deep hypothermia. Since the ventilation rate remained high relative to the oxygen consumption even to the stage of terminal apnea, the progressive fall in oxygen consumption cannot be ascribed to a paralysis of external respiration.

Fig. 3. RELATIONSHIP BETWEEN OXYGEN CONSUMPTION ($\text{mm}^3/\text{min}/\text{gm. body weight}$) and colonic temperature before, during and after cooling of 6 guinea pigs.

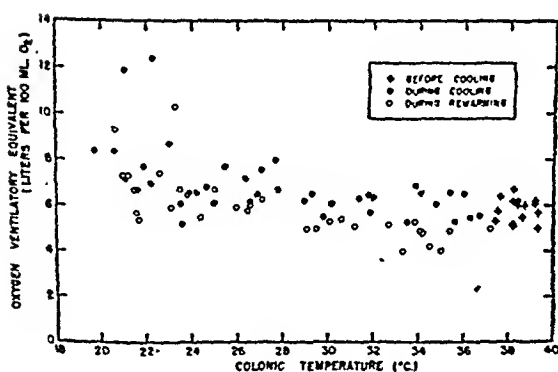
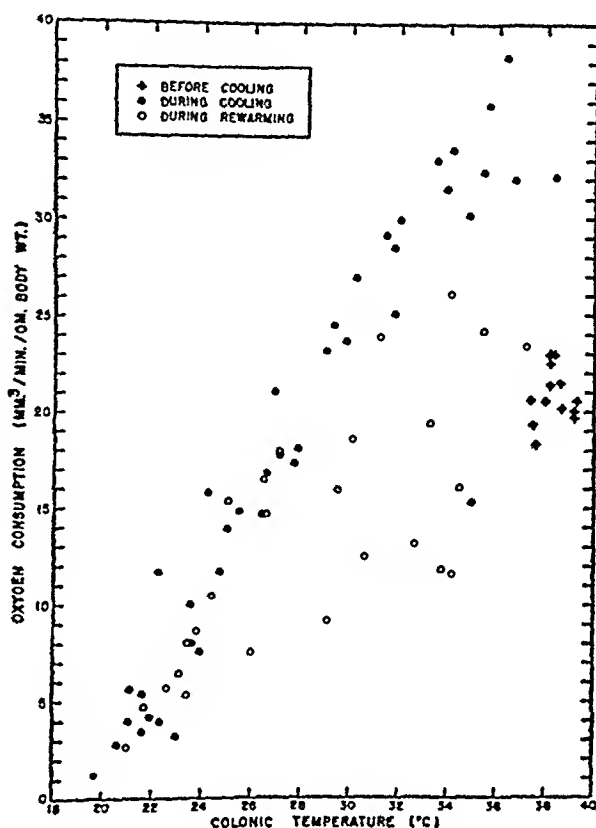


Fig. 4. RELATIONSHIP BETWEEN OXYGEN VENTILATORY EQUIVALENT ($\text{l}/100 \text{ ml. oxygen consumed}$) and colonic temperature before, during and after cooling of 6 guinea pigs.

In mild prolonged hypothermia in man, Dill and Forbes (4) report a fall in respiratory quotient (RQ) and suggest that a depletion of liver glycogen is responsible. In the present study, a correlation between RQ and colonic temperature cannot be demonstrated unequivocally. A trend of questionable significance consisted of an elevation of RQ during the early phases of cooling, and a return toward the pre-cooling average (0.85) in deep hypothermia. Late in recovery, the RQ distinctly

fell as the body temperature returned toward normal. These shifts may indicate no more than a depletion of carbohydrate reserves.

During rewarming the ventilation rate and oxygen uptake slowly rose, but both rates were lower than those during cooling (at the same colonic temperature). The difference is probably related in part to the development of fatigue and decrease in struggling. Except for heart rate, vital signs during rewarming varied widely among the tests. The oxygen consumption (fig. 3) and ventilation rate may rise so slowly that pre-cooling values are never reached, may return to pre-cooling values in mid-recovery (about 27°C.) and then stabilize as the temperature continues to rise, or may climb late in mid-recovery to slightly above pre-cooling rates and then return to initial values. Of course, those animals with the most intense metabolism restored normal body temperatures fastest. Among the others, late fatalities were frequent, as demonstrated below. Early in the rewarming, however, it was impossible to predict which of the above metabolic patterns would develop. A consistent trend during recovery was a steady decline in the oxygen ventilatory equivalent (fig. 4) to levels 10 to 40 per cent below pre-cooling values. Data are inadequate to appraise the functional significance of this decline or to decide whether it is less marked among survivors than among those who die in mid-recovery.

Figure 5 summarizes observations on the colonic temperature, heart rate, ventilation rate, oxygen consumption and oxygen ventilatory equivalent during a typical cooling and the early phases of rewarming.

Survival. In analyzing survival, it is convenient to establish four categories of performance as in table 1. Insofar as sampled in these tests, sex and body weight did not condition performance. Acclimatization did not modify the average result since only rarely was the same animal used in more than one experiment. The average minimal temperatures are recorded in the table only to demonstrate that this factor did not determine the outcome, since these averages do not differ appreciably.

Immediate deaths (class A in the table) are those which occurred during cooling or shortly thereafter (within 1°C. of the minimum). As was intended, such fatalities were few. Even if the samples were larger, such data could not be interpreted in terms of a median lethal temperature, because the duration of exposure at any one temperature was not controlled and death cannot be associated with any particular colonic temperature when the latter is changing rapidly. Finally a hypothermic death can be detected only by the animal's failure to revive in a specified post-exposure environment. There is no test of immediate applicability to prove that a hypothermic injury is irreversible. A possible exception is electrical asystole of the heart, which in these tests proved incompatible with recovery however abetted. On the other hand, Fairfield (13) reports spontaneous recovery of infant rats restored to air at 35° after electrical asystole for 1 hour or more.

From these data one may conclude tentatively that at this rate of cooling no guinea pig's colonic temperature can be reduced below 21°C. with impunity and that a prompt death is almost certain when the temperature falls to 17°C. Without indicating the depth of their thermocouples, Weltz, Wendt and Ruppel (14) report that in guinea pigs colonic temperatures of 15°C. for 15 minutes, 18°C. for 1 hour,

or 25 to 30°C. for about 10 hours are just compatible with survival. Such limits are lower than those suggested by the present data. When compared with results on rats (15) and on rabbits (16), the guinea pig appears to be especially susceptible to a hypothermic death. The mechanisms which led to these prompt fatalities remain obscure. Although no complete studies of ventilation and heart rate were done on

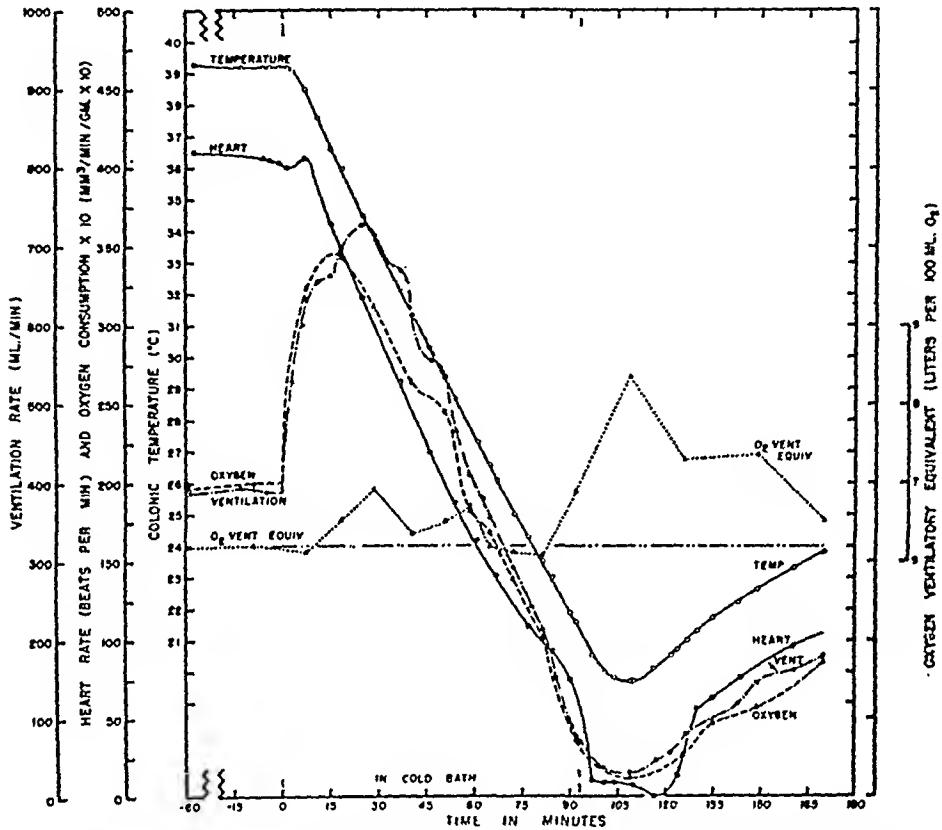


Fig. 5. OBSERVATIONS ON A SINGLE GUINEA PIG during cooling and early rewarming in room air (27.5° C.). Jacketed animal in ice bath between times 0 and 93 (min.).

TABLE I. ANALYSIS OF SURVIVAL

CLASS	MEAN MINIMAL COLONIC TEMP. (± S. D.)	NO. OF ANIMALS	PER CENT OF TOTAL
	°C.		
A Immediate deaths.....	(18.7)	5	18
B Death after partial recovery.....	18.7 ± 0.8	7	25
C Survivals.....	19.4 ± 1.2	14	50
D Atypical deaths.....	19.2	2	7
TOTAL.....		28	100

these particular animals (class A) there is no reason to suspect that their responses to hypothermia were qualitatively different from those of the survivors. As previously demonstrated a failure of external respiration is not the limiting factor since relative to the oxygen uptake, the ventilation rate remained high up to the stage of terminal apnea. The latter appeared at about the same time as a precipitous drop

in heart rate, but electrical systoles generally outlasted any detectable breathing by several minutes. On the other hand, terminal deterioration is so rapid, profound and generalized that a satisfactory explanation of a hypothermic death can hardly be expected from studies of the relative times at which respiratory and cardiovascular collapses appear. Crismon (1) believes that death in cooled rats is due to cardio-circulatory failure as evidenced by an extreme drop in heart rate and in arterial blood pressure. Such an interpretation offers a tentatively acceptable explanation of the immediate deaths in the present study. In accord with previous reports, pertinent findings on post-mortem examination were few. Not infrequently, there was engorgement of the great veins, of the right heart, and sometimes of the pulmonary vascular bed, but pulmonary edema, adrenal hemorrhage and adrenal enlargement were not apparent on gross inspection.

The animals that showed a partial recovery and delayed death (class B in the table) are of particular interest. When restored to room air, their temperatures climbed slowly but steadily; their heart rates accelerated in a manner consistent at all times with their increasing colonic temperature; ventilation rates rose; spontaneous motor activity returned. Some of these animals made much faster progress in repaying their thermal debt than others, but, whatever their accomplishment, colonic temperatures tended to level off after 3 to 5 hours when shivering and struggling slackened. This left some animals with body temperatures only slightly below normal, while others were stranded at distinctly hypothermic levels. The latter were often unable to support their own weight or to take food, but this was not invariably true.

All animals were returned to individual cages. The next morning some were dead. With only two exceptions the fatalities occurred among those animals which had not succeeded in regaining colonic temperature of 30°C . within 4 hours after their immersion. By extrapolating the temperature curves of these animals who died within 10 to 15 hours of the immersion (class B), it becomes apparent that none of them succeeded in re-establishing a normal colonic temperature. Most of them ceased to warm while under observation and at colonic temperature ranging from 27.5 to 30.5°C . Among survivors (class C), either the body temperature continued to rise throughout the 4 to 5 hours of observation or it stabilized above 34°C .

It is apparent that the damage sustained during severe chilling impairs a guinea pig's ability to repay its thermal debt. In this respect, its performance is critically influenced by the environmental temperature during rewarming. Of 14 animals rewarming in still air at 25°C ., 6 failed to restore a normal colonic temperature and eventually died; at 27.5°C . the environment overtaxed the restorative capacity of only one animal in 7. Can one predict at the end of the cooling which animals have received a fatal insult and will prove incapable of regenerating a normal body temperature? Within broad limits this damage cannot be estimated from the intensity of the exposure as measured by the minimal temperature reached during cooling (table 1). Electrocardiograms and studies of ventilation and of oxygen consumption during cooling do not pre-assess this damage. Indeed one animal, which eventually recovered completely and promptly, was apneic for 20 minutes shortly after cooling. Even early in the rewarming operation, oxygen consumption and ventilation rate

do not sufficiently distinguish between animals of class B and C to be of prognostic value. For predictive purposes, the vital signs studied here are inadequate.

The delayed deaths (class B) cannot be explained any more satisfactorily than they can be predicted. Probably the cooling contributed only by so impairing thermoregulation that thereafter some animals could maintain body temperature only a few degrees above that of room air. Prolonged exposure to mild degrees of hypothermia (from 28 to 31°C.) was the essential feature of these deaths. Weltz *et al.* (14) observed that with colonic temperatures held at 25 to 30°C. his guinea pigs died after about 10 hours. That a hypothermic injury may cause death after com-

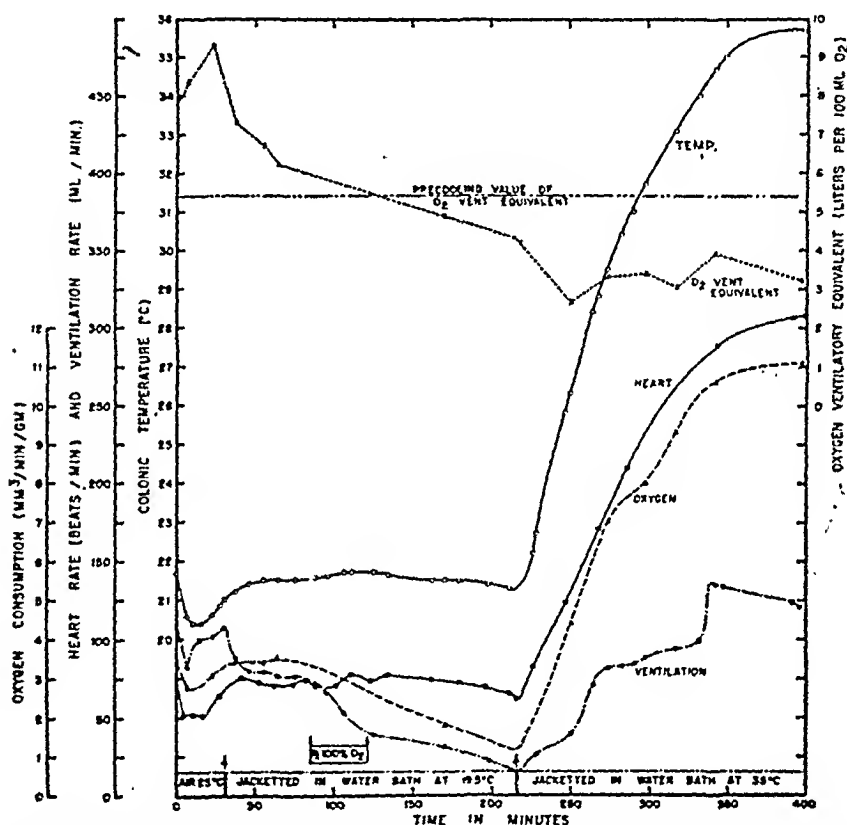


Fig. 6. OBSERVATIONS ON A JACKETED GUINEA PIG removed from ice bath at zero time, held in a cool bath for over 3 hours, and then rewarmed in a bath at 35°C. Between times 85 and 121, pure oxygen was inspired.

plete spontaneous thermal recovery has been reported occasionally but was not demonstrated in the present study.

Delayed deaths after partial recovery (class B) did not occur during the period of experimental observation. Probably the same functional disturbances occurred in more accelerated fashion when an animal was placed in a cool post-immersion environment. A thermal equilibrium was then rapidly established, and the body temperature stabilized at a value which could not be tolerated long. As illustrated in figure 6, this was accomplished by allowing a jacketed animal to rewarm in a water bath at 19.5°C. The colonic temperature promptly leveled off at about 2°C. higher. The heart rate remained constant for almost 3 hours when a downward trend began. The ventilation rate fell steadily but slowly throughout; on other occasions, it too

stabilized for over 2 hours before the inevitable decline. During such an isothermal period, alterations in heart rate were invariably preceded by a severe and sustained depression of respiration. As long as the body temperature was held constant, any downward trend in heart rate was found to signal an impending death, and this sign was thought to be more reliable prognostically than the absolute values of heart rate, ventilation rate, or oxygen consumption. It is obviously difficult to ascribe such a death to a primary cardiovascular collapse, however tenable this interpretation of the prompt fatalities in severe hypothermia (class A). As illustrated in figure 6, relative to the fall in oxygen consumption the diminution in ventilation rate was not critical. Indeed if these delayed deaths are due to cumulative anoxic damage, the primary defect does not appear to be in alveolar ventilation or in the transport of blood. Such experiments make it doubtful that any one factor is uniquely critical in all hypothermic situations.

In any case the type of deterioration illustrated in figure 6 is relatively slow and probably susceptible to appropriate supportive measures. Of the many measures suggested (2, 9, 10), only two were tested. The inhalation of pure oxygen did not modify the vital signs at any time and did not forestall their decline. The only measure of apparent benefit was vigorous artificial rewarming. In the experiment of figure 6, this was accomplished by immersing the jacketed guinea pig in a water bath held at 35°C. The restoration of temperature and of vital signs was prompt. Although the oxygen consumptions rose considerably faster than the ventilation rate, the latter was not obviously inadequate. However the ventilation rate, oxygen uptake and oxygen ventilatory equivalent at the end of the test were all appreciably lower than in animals which recovered spontaneously. It is worthy of emphasis that by all previous experience this animal was doomed by his protracted exposure and that in room air he would have survived no more than a few hours. But the low ventilatory and metabolic response seen here suggest a danger possibly inherent in rapid artificial rewarming.

SUMMARY

Observations were made on 30 unanesthetized mature guinea pigs during and after severe reductions of body temperature produced by immersing the jacketed animals in ice water. Measurements included colonic temperature, electrocardiograms, ventilation rate (minute respiratory volume), oxygen consumption, and carbon dioxide production. Regardless of the rate of cooling or rewarming, heart rates varied linearly with colonic temperatures between 23 and 35°C., falling 17.5 beats per minute for each decrement of one Centigrade degree. Below 23°C. the drop was erratic and often precipitous. Sinus and nodal arrhythmias, premature beats of auricular and ventricular origins, auriculoventricular blocks, and reversible ventricular fibrillation were all noted.

The early phases of cooling were characterized by increased struggling, hyperpnea, enhanced metabolism, and cardiac slowing. When cooling at 0.2 to 0.3 degrees per minute, maximal ventilation occurred at 35°C. (33° to 37°) and averaged 170 per cent of the pre-cooling rate. At this point maximal rates of oxygen consumption also amounted to 170 per cent of the pre-cooling level or 250 per cent of the

estimated basal. Below 33°C., ventilation and oxygen consumption diminished progressively with temperature, dropping below pre-cooling rates between 26 and 29°C. and approaching zero slightly below 20°C. Relative to the oxygen uptake, however, the ventilation rate (expressed as the oxygen ventilatory equivalent) remained high to the stage of terminal apnea. Therefore, the progressive fall in oxygen consumption cannot be ascribed to failure of external respiration.

Some animals survived briefly at 17.5°C., others succumbing at as high as 21°C. This large variation in lethal temperature is unexplained. The mechanisms of a hypothermic death remain obscure, but these data suggest that in severe chilling critical circulatory inadequacy often precedes respiratory failure.

Guinea pigs which did not die promptly in deep hypothermia were allowed to rewarm in room air. During rewarming ventilatory and metabolic rates varied widely among the tests, and the temperature of some animals rose much more slowly than that of others. It is postulated that damage sustained during severe chilling impairs the capacity to repay a thermal debt. It was not possible to predict final rates of recovery from vital signs during cooling or even during the early phases of rewarming. Beyond 4 to 6 hours after the immersion, relatively little progress was made in raising the body temperature, leaving some animals stranded at distinctly hypothermic levels (about 30°C.). The latter died within 10 to 15 hours of the immersion. Slight departures of the heart rate from predictions based on the colonic temperature appeared to signal such impending deaths. Whatever the derangements responsible for these delayed fatalities, it seems probable that they represent controls different from those operating at the lethal temperature during cooling.

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ANALGESIA AND ANESTHESIA INDUCED BY EPINEPHRINE

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EARLIER investigations (1) have shown that the intracisternal (i.c.) injection of epinephrine (epn) in large doses induces analgesia, drowsiness and sometimes sleep. These observations are in accord with the findings of Ivy (2) and co-workers, namely, that epn injected into the carotid arteries of dogs produces analgesia. On the basis of these observations, experiments were performed to evaluate the intrathecal injection of epn for surgical anesthesia.

METHODS

Twenty-six experiments were made on dogs. In the first 2 experiments, epn solution (Winthrop) was employed, but in the remainder, epn-base-powder (Parke-Davis) was used to eliminate the preservatives, sodium bisulfite and chloro-butanol, which perhaps might contribute to the anesthesia. The epn-powder was dissolved in 1 or 2 cc. sterile, distilled water and slightly acidified with diluted HCl. Two or three cc. of cerebrospinal fluid (CSF) were removed and discarded. The epn-solution, placed in a syringe, was diluted by directly and repeatedly withdrawn CSF (barbotage). Due to the alkalinity of the CSF, the pH of this mixture, as seen in repeated tests, was approximately 7.4. The diluted epn-solution was then injected i.c. The volume injected was always adjusted to approximate the volume of CSF originally withdrawn. At the end of the injection, a small amount of CSF was again drawn into the syringe to demonstrate that the entire injection was intracisternally. When the CSF was blood-tinged no experiment was performed.

In 6 experiments, epn alone ($\frac{1}{2}$, or 1 mg/kg.) was injected i.c. without any preliminary sedatives. In another group of experiments, small amounts of nembutal (20 mg/kg.) were injected intraperitoneally (i.p.) as basal anesthesia (b.a.). This was done in order to decrease the amount of epn necessary to produce anesthesia and to make the i.c. injection a less difficult procedure. Several hours after the nembutal injection, when the dogs were awake, epn ($\frac{1}{2}$ mg/kg.) was injected i.c.

In 3 other experiments, ephedrine sulfate (in the amount of 5 mg. or 15 mg/kg.) was given i.c. with preliminary b.a. In a few experiments, NaCl-solutions (0.9% and 15%) were injected i.c., also with previous b.a. and in one experiment procaine-HCl (in the amount of 6 mg/kg.) was administered i.c. without preliminary sedation.

In most of these experiments, the blood pressure (by way of a cannula from the femoral artery), the respiration (by means of a pneumograph) and the electrocardiogram (ECG, by a directly writing apparatus, Sanborn,) were recorded. In a few experiments, electroencephalographic records (EEG, by means of a Grass-amplifier and inkwriter) were taken.

In another group of experiments (including 11 guinea pigs and 1 dog), epn in different concentrations was injected intracutaneously (into the outer side of the right hind leg): 1 cc. of 1:30000 (9 guinea pigs and one dog); 1 cc. of 1:40000 (2 guinea pigs).

For control, 1 cc. of physiological NaCl was injected intracutaneously into the corresponding area of the opposite hind leg.

RESULTS

Action of i.c. epn. When epn alone ($\frac{1}{2}$ or 1 mg/kg.) had been injected the dogs became quiet within 10 to 15 minutes (following a brief period of excitement) and 30

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minutes after injection they were asleep. Sleep occurred earlier when the head of the dog was maintained in a lower position than its body. The sleep lasted for 1 to 2 hours. The respirations were deep and regular all this time. About $\frac{1}{2}$ hour after the onset of sleep, pinching, cutting or suturing the skin elicited no signs of pain from the dogs (i.e. restless movements of the legs, growling or whining). To perform a laparotomy an additional amount of epn ($\frac{1}{2}$ mg/kg.) had to be injected i.c. about $\frac{1}{2}$ hour after the previous injection. Following this, a complete laparotomy did not result in any evidence of pain. Specifically, the abdominal muscles could be cut, the

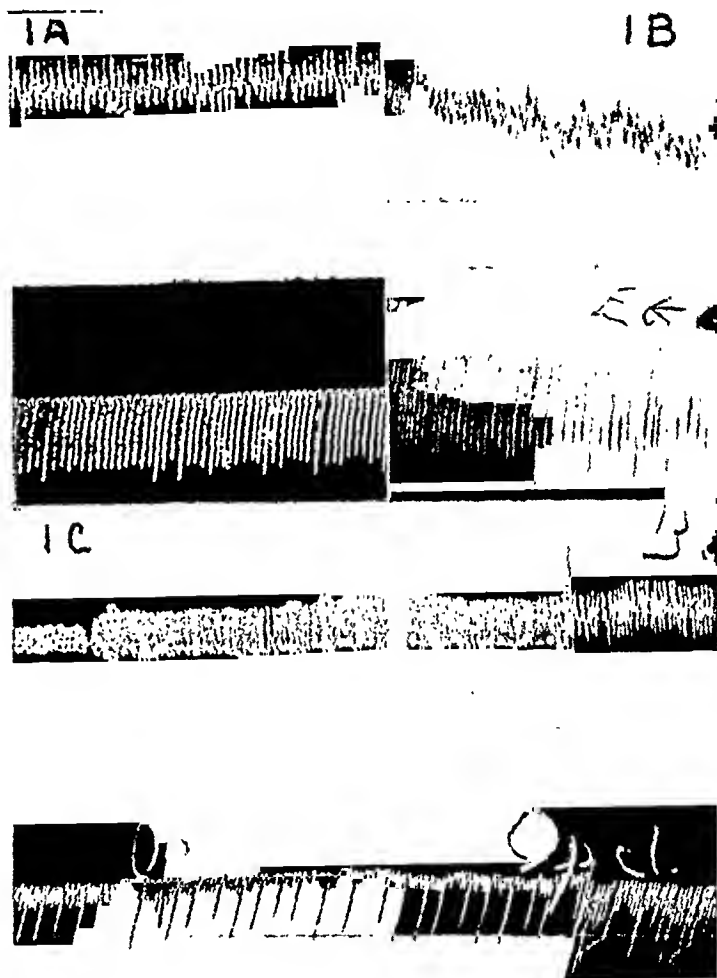


Fig. 1. DOG 11 KG. *Upper tracing: blood pressure; lower tracing: respiration.* (A) before injection; (B) after i.c. injection of 5 mg. epinephrine (powder). (C) DOG 15 KG.; *upper tracing: blood pressure, lower tracing: respiration during a laparotomy after i.c. injection of 15 mg. epinephrine (powder).*

peritoneum incised and traction could be exerted on the peritoneum and mesentery. The degree of relaxation of the abdominal muscles was complete.

Usually 4 hours after the nembutal injection, in the second group of the epn experiments, the animals were fully awake so that they struggled to free themselves from the animal board and gave distinct signs of pain on pinching the skin. Epn ($\frac{1}{2}$ mg/kg.) was then injected i.c. After a short period of excitation, the dogs fell asleep within 10 minutes and remained in sleep for 3 to 4 hours. Again the onset of sleep was hastened by lowering the dog's head. Approximately, 30 minutes after the injection, complete surgical anesthesia occurred. In 7 such experiments, laparotomies were performed without any evidence of pain on the part of the dog.

The blood pressure, observed during the entire experiment, even during a laparotomy, remained at the normal level (fig. 1A, B, C). The ECG also remained without any change (fig. 2A, B). In contrast, the ECG of the same dog showed many ventricular extrasystoles after intravenous injection of 1 mg epn (fig. 2C, D) and the typical increase in blood pressure occurred.

Electroencephalographic studies revealed no significant change after i.c. epn in the doses which produced anesthesia. Only after the administration of extremely large doses ($2\frac{1}{2}$ to 3 mg/kg.) were the EEG-waves greatly depressed in voltage. Later the electrical activity of the brain ceased. At the same time, the ECG showed ventricular fibrillation and the dog died. The injection of these tremendous amounts of epn involved changes which could be attributed to the effects of hypertonicity, as

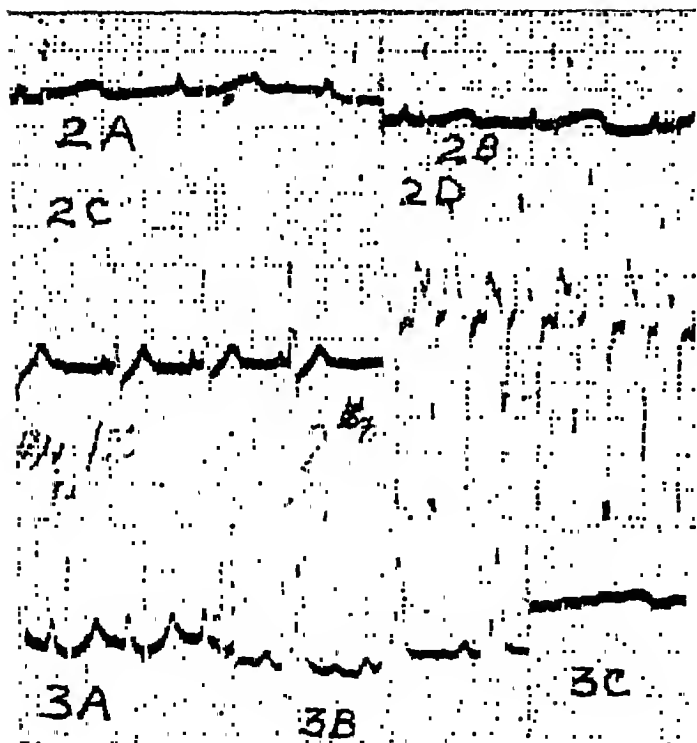


Fig. 2. DOG 14 KG. ECG: (A) before injection; (B) after i.c. injection of 8 mg. epinephrine (powder). Same dog ECG: (C) before injection; (D) after i.v. injection of 1 mg. epinephrine (powder).

Fig. 3. DOG 10 KG. (A) ECG before injection; (B) ECG after i.c. injection of ephedrine sulfate 15 mg/kg. (note high anoxic T-waves); (C) ECG 10 minutes after i.c. injection of ephedrine sulfate 15 mg/kg. (note ventricular fibrillation).

will be explained later. After i.c. epn, the respiration was not depressed; on the contrary it was usually stimulated, mainly by increase in amplitude (fig. 1A, B).

It should be mentioned that sometimes, especially in the experiments without nembutal, a transitory rigidity of the forelegs and of the neck appeared shortly after the i.c. injection of epn. This stiffness disappeared completely on the following day.

All the dogs in the above-mentioned groups except two (into which excessively large doses of epn ($2\frac{1}{2}$ –3 mg/kg.) had been injected purposely) were in good condition the day following the i.c. injection, even though a laparotomy had been performed. They remained so during the time of observation (2–4 months). No after-effects were seen during this time, even though, in some cases, 3 or 4 i.c. injections of epn, at various intervals, had been made into the same dogs.

Action of i.c. Injection of Ephedrine Sulfate. Following i.c. ephedrine (5 mg/kg.)

no analgesia and no tendency to sleep could be detected (in 2 experiments). The blood pressure rose from 130 to 200 mm. Hg and remained at this high level for the time of observation ($1\frac{1}{2}$ hours). The ECG showed many ventricular extrasystoles and later high anoxic T-waves. After about 40 minutes normal ECG-waves re-appeared. In one experiment, after i.c. ephedrine 15 mg/kg., likewise no evidence of analgesia or sleep could be observed. The blood pressure rose from 130 to 210 mm. Hg and 20 minutes later fell suddenly to zero (fig. 3). The ECG revealed severe changes, many ventricular extrasystoles, high anoxic T-waves and finally ventricular fibrillation with resulting death of the dog (fig. 3A, B, C).

Action of i.c. Procaine-HCl. In one experiment, following procaine HCl (6 mg/kg.) pronounced dyspnea and severe cyanosis appeared almost immediately. No anesthesia to pricking or pinching was found. No sleep occurred. A transient paresis of the hindlegs lasting one hour was observed (the dog had been placed in Fowler's position). Within $1\frac{1}{2}$ hours the dyspnea and cyanosis disappeared.

Action of the Intracutaneous Injection of Epn. All the guinea pigs and the one dog showed analgesia to pricking, pinching and cutting 10 to 15 minutes following the intracutaneous injections of 1:30,000-1:40,000 epn. This analgesia lasted from 24 to 48 hours. No necrosis was seen at the site of the injection. The sites of saline-injection revealed no analgesia or only temporary hypalgesia for a few minutes.

DISCUSSION

The fact that general anesthesia and sleep can be induced by i.c. epn indicates that this substance exerts an effect on some center or centers in the brain (perhaps hypothalamus or brain stem). The question may arise whether the observed phenomena might be attributed to vasoconstriction following i.c. epn administration. Such an assumption does not hold. Fog (3) Forbes (4) and co-workers have shown that the direct application of epn to the pia mater produces no constriction of the arterioles and only slight short-lasting constriction of the large arteries. In our experiments, however, sleep and analgesia lasting several hours were produced.

There is other evidence of the effect of epn on the central nervous system without concomitant vasomotor action. In our previous experiments (1) it was demonstrated that epn injected intrathecally causes a rapid, high sustained rise in blood sugar without changes in the blood pressure and the ECG. The analgesic effect of intracarotid injection of epn in dogs, shown by Ivy (2) and co-workers, suggests a direct action of epn upon pain perceiving centers. Further, the recently reported thermogenetic effect of epn on the brain (Pick and Feitelberg) (5) reveals a central action of epn without accompanying blood pressure effect.

From our present experiments, we conclude that epn has central analgesic and anesthetic properties. Moreover, the long-lasting local anesthesia after intracutaneous administration of epn suggests that epn has a direct effect on peripheral nerves independent of vasomotor action and is probably capable of blocking nerve conduction.

In this connection it might be mentioned that recently reported experiments seem to demonstrate that there exists a relationship between morphine-analgesia and epn. The analgesic response to morphine of rats (6) and dogs (7) was markedly reduced

after removal of the adrenal medulla. Other earlier experiments have shown that morphine causes the release of epn from the adrenal medulla (8).

With regard to the question, whether the i.c. injection of the above-mentioned large doses involves some danger to the cardiovascular system, there might be said the following. It was demonstrated in earlier experiments that epn injected intrathecally does not raise the blood pressure (1/10/11). The same was seen in these experiments during which much larger doses were employed.

In order to avoid a rise in blood pressure, it is necessary to take three precautions: 1) Epn must never be injected i.c. when the withdrawn cerebrospinal fluid is bloody or blood-tinged; otherwise epn can enter the general circulation. 2) I.c. injection of epn should be made only after removal of a volume of CSF equal to the volume of fluid to be injected. 3) A hypertonic solution of epn should never be injected. This can be avoided by dilution of the epn-solution in the syringe through repeated mixing

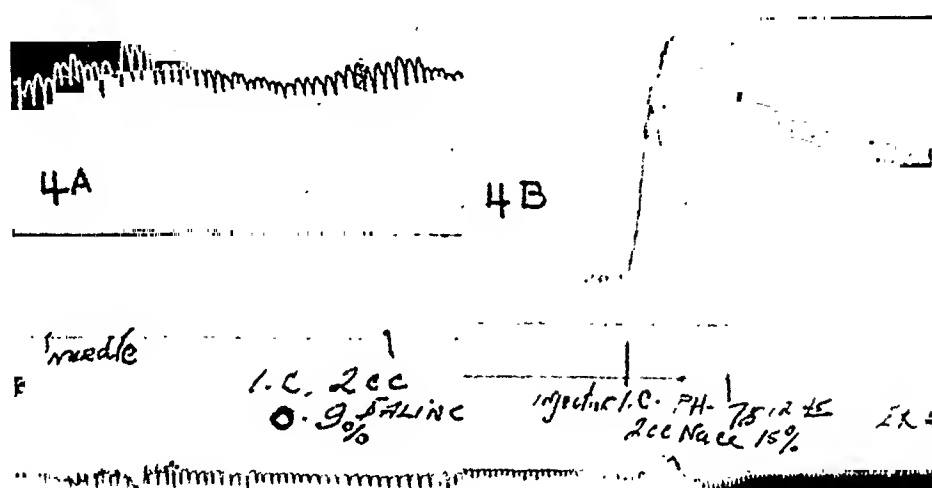


Fig. 4. DOG 11 ECG. Nembutal anesthesia. Upper tracing: blood pressure; lower tracing: respiration. (A) after i.c. injection of 2 cc 0.9% saline pH 7.4; (B) after i.c. injection of 2 cc NaCl 15%.

with CSF (barbotage). Further, a large amount of epn should not be injected i.c. at one time, but in small fractions at proper intervals. When these precautions are taken the ECG does not change either. In this connection, it should be pointed out that a hypertonic NaCl-solution itself, injected i.c. can provoke a pronounced rise in blood pressure and severe changes in the ECG (fig. 4).

Of great importance is the fact that the respiration is not depressed after i.c. epn, but on the contrary is stimulated (probably by direct stimulation of the respiratory center). Crystalline epinephrine base seems to be more effective than other epinephrine preparations.

Another sympathomimetic drug, ephedrine sulfate, i.c. does not produce analgesia nor sleep in the dog. Nor has procaine-HCl an analgesic effect after i.c. injection in contrast to its well-known action after lumbar injection. There exists a tendency among anesthesiologists to use epn (11, 12) or ephedrine (13, 14) as an adjunct in procaine spinal anesthesia. Our experiments suggest that epn might aid in this analgesia not by ischemia, but synergistically as an analgesic. Caution

should be exercised in the intrathecal injection of ephedrine as an adjunct in spinal anesthesia.

SUMMARY

Intracisternal injection of epinephrine alone ($\frac{1}{2}$ –1 mg/kg.) produces surgical anesthesia in dogs. An additional amount of epn ($\frac{1}{2}$ mg/kg.) is necessary to accomplish complete surgical anesthesia for laparotomy. When small amounts of nembutal are injected intraperitoneally several hours prior to i.c. epn, a smaller amount of epn ($\frac{1}{2}$ mg/kg.) is adequate for complete surgical anesthesia. The blood pressure, ECG and the EEG remain normal after i.c. epn in the mentioned doses. The respiration is not depressed but stimulated (mainly in amplitude). No after effects are observed. Excessively great doses of i.c. epn (about $2\frac{1}{2}$ –3 mg/kg.) are toxic. The possibility of extending these investigations for use in operation on man is discussed.

The i.c. injection of ephedrine sulfate or procaine-HCl does not produce analgesia or sleep in the dog.

The authors thank Mr. G. Warner for his technical assistance.

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MEASUREMENT OF EXPERIMENTALLY INDUCED BRAIN SWELLING AND SHRINKAGE¹

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IN STUDIES of the mechanisms of cerebral edema and swelling a simple method for estimating the extent of changes in brain volume in experimental animals is needed. In the present paper it is shown that determinations of dry weight provide a simple method for estimating variations in brain tissue volume, and some observations on swelling and shrinkage are described.

MEASUREMENT OF CHANGE IN BRAIN VOLUME

White *et al.* (1, 2) have measured changes in brain volume in cats by careful determinations of the volume of the brain and of the cranial cavity. The normal difference between these two volumes is about 10 per cent and variation from this figure represents swelling or shrinkage of the brain. This method is difficult, and could hardly be applied after craniotomy. Assuming that changes in brain volume are due to changes in water content, and that the percentage of dry matter in the brains of normal animals is constant, then a difference from normal in the percentage dry weight of the brain of an experimental animal must be a measure of a change in volume. The swelling or shrinkage may be simply calculated without any need to determine the actual volume of the brain.

If W and D are respectively the fresh and dry weight of the brain of a normal animal and P is the percentage dry weight, then $W = D \times \frac{100}{P}$. If, as a result of treatment, the weight and percentage dry weight change to W_1 and P_1 , then $W_1 = D \times \frac{100}{P_1}$. Whence $W_1 = \frac{P}{P_1} W$. The swelling or percentage change in weight (or volume, since the tissue density is about unity) is given by swelling percentage = $\frac{W_1 - W}{W} \times 100 = \frac{P - P_1}{P_1} \times 100$.² It should be noted that presentation of results

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² If the swelling or shrinkage is caused by the absorption or loss of fluid which itself contains some solid matter, then a larger change in volume would correspond to a given change in percentage dry weight. If p is the percentage dry weight in the fluid absorbed or lost, it can be shown that

$$W_1 = \frac{P - p}{P_1 - p} W \text{ and percent swelling} = \frac{P - P_1}{P_1 - p} \times 100.$$

in terms of percentage moisture, as is commonly done, rather than dry weight, tends to give a misleading impression. If the normal moisture content is 80 per cent, a change to 81 per cent indicates a 5 per cent increase in weight since $W_1 = \frac{20}{19} W = 1.05W$.

DETERMINATION OF DRY WEIGHT

Rabbits were anesthetized with Nembutal and decapitated. The roof of the skull was removed and the cerebrum was removed from the skull by cutting through the mid-brain along the bony edges of the incisura of the tentorium. This method was adhered to carefully so that the parts of the brain treated, particularly with respect to the relative proportions of grey and white matter, were always the same. The cerebrum was halved midsagittally and the determination was carried out on one half, or on both halves separately as duplicates. All free fluid was carefully wiped out of the ventricles with filter paper. The whole hemisphere was then pushed into a tared weighing bottle with a helmet-type cover and containing a short sealed glass tube with a mace-like head (figure 1). This process was done rapidly, or in a humid chamber, since loss of moisture by evaporation could be appreciable. After determining the fresh weight of the hemisphere, 2 ml. of acetone were run into the bottle and the tissue was carefully reduced to a suspension by mashing with the 'mace'. The acetone was then evaporated away by directing a current of filtered air into the bottle. When the tissue could be spread as a paste around the sides of the bottle, it was placed in an oven at about 108° for 24 hours or more, cooled in a desiccator and weighed. Some care was necessary to insure that foaming of residual acetone did not cause loss of material when the temperature was first raised. After 24 hours, further loss in weight was negligible. Without the acetone treatment, complete drying took much longer. There was never any increase in weight, as has been reported for some fatty tissues on prolonged heating, following this method of acetone treatment.

The agreement between values obtained for the percentage dry weight of left and right hemispheres from the same animal was good. In 25 such pairs of determinations the widest difference was 0.3; usually the difference was much less. However, rather wide variations between individual animals were found (table 1), which could not be correlated with depth or period of anesthesia, weight, or method of killing (bleeding, decapitation, or constriction of the neck).

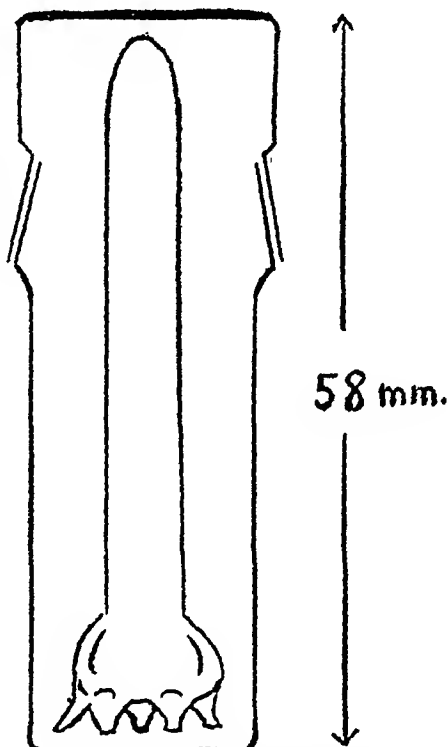


Fig. 1. WEIGHING BOTTLE WITH MACE for determining the dry weight of brain. Larger bottles could be used for brains of larger animals.

The average value for normal animals was 21.16 per cent³ with a standard deviation of the distribution equal to 0.5. In view of the range of variability of the normal animals, values for brains of individual experimental animals between 20.2 and 22.1 per cent cannot be regarded as significantly different from normal. That is to say, a change of less than 4 per cent in brain volume, calculated on the basis of the average normal percentage dry weight, cannot be reliably ascribed to the treatment of the animal.

While this work was in progress a paper by Windle *et al.* (3) appeared in which a similar method for determining brain dry weight was described. This method appears to be extremely accurate but somewhat more cumbersome than the present method. The tables of Windle *et al.* show that the dry weight content of normal guinea pig brains varied between 20.5 and 21.7 per cent, a range of variability approaching that found by us for rabbits. Windle *et al.* found a statistically significant increase of 0.5 per cent in the mean moisture content of brains from concussed animals and an increase of 0.7 per cent with animals which had been water-logged by stomach tube. It was concluded that edema following concussion is significant but slight. Calculation shows that an 0.5 per cent increase indicates about 2.5 per cent swelling which is appreciable if the available space is only 10 per cent. In earlier work Pilcher (4, 5) attempted to detect changes of brain water in traumatized dog brains, by determinations of dry weight in various parts of the brain. His tables also show great variability; values were reported for the dry weight content of cerebral grey matter between 20.1 and 21.7 per cent in 5 normal animals and between 18.7 and 22.5 per cent in unexposed sides of unilaterally exposed brains. Average figures indicated only slight, though definite, increases in moisture content following trauma with the skull intact and none with the skull exposed.

The variability of the percentage dry weight of normal brain seriously limits the precision of determinations of swelling or shrinkage by the dry weight method. Donaldson (6, 7) in extensive studies on rats, has shown that the moisture content of the brain is affected in a regular manner by the age and size of the animal and by the size of its brain. Even with animals from the same age group there is rather wide variability (s.d. 0.2 to 0.5) but this variability is considerably less among litter mates (s.d. 0.13).

EXPERIMENTALLY INDUCED CHANGES IN BRAIN VOLUME

Weed *et al.* (8-12) showed that considerable changes in brain bulk and spinal fluid pressure could be produced in cats by intravenous injections of hypo- and hypertonic fluids. Similar procedures have been used here. Portions of the skulls of rabbits, anesthetized with Nembutal, were removed and the dura reflected to expose the brain. Either 0.1 per cent glucose solution (hypotonic fluid) or 25 per cent glucose (hypertonic fluid) was infused into the femoral vein, at a rate of about 2 ml. per minute, usually for 60 to 75 minutes. Sometimes the fluid was infused into the

³ It should be noted that the value, 21.16 per cent, for the average dry weight content has no absolute significance since it represents only a particular mixture of grey and white matter from a variety of brain regions. It is of value only for comparison with similar brain samples from different animals.

internal carotid artery but the results were about the same. The percentage dry weight of the brains of animals thus treated and the change in brain volume calculated therefrom are shown in table 2.

Hypotonic infusion caused definite swelling of the brain tissue. Volume changes were found between 6 and 15.5 per cent, calculated on the basis of average normal dry weight. Hypertonic infusion caused very marked shrinkage, up to 31 per cent in the case of one animal infused for 3 hours. The shrinkage of the brain relative to the cranium was very obvious in all cases of hypertonic infusion and noticeable within 20 minutes of starting the infusion. Chemical determinations on the brain of one of these animals showed that excess of glucose or lactate in the brain could not account for an appreciable fraction of the increased percentage dry weight.

TABLE 1. VARIATION IN DRY WEIGHT CONTENT OF NORMAL RABBIT BRAINS

% dry weight.....	20.1	20.3-20.5	20.6-20.8	20.9-21.1	21.2-21.4	21.5-21.7	21.8-22.1	22.3
No. of animals.....	1	0	4	7	6	2	2	1

TABLE 2. EFFECTS OF INTRAVENOUS INFUSION OF HYPO- AND HYPERTONIC FLUIDS ON PERCENTAGE DRY WEIGHT OF BRAIN

HYPOTONIC INFUSION		HYPERTONIC INFUSION		HYPOTONIC INFUSION		HYPERTONIC INFUSION	
Per cent dry weight	Per cent ¹ swelling	Per cent dry weight	Per cent ¹ swelling	Per cent dry weight	Per cent swelling	Per cent dry weight	Per cent swelling
<i>Brain exposed</i>				<i>Skull intact</i>			
19.1	+11	23.6	-10.5	19.85	+6.5	21.85	-3
20.0	+6	24.7	-14.5	21.05	+0.5	22.4	-5.5
18.5	+14.5	26.7	-21	21.5	-1.5	23.4	-9.5
18.3	+15.5	24.6	-14	20.35	+4	23.4	-9.5
19.7	+7.5	30.8 ²	-31	19.5	+8.5		
19.5	+8.5			21.35	-1		
19.2	+10						

¹ Calculated on the basis of 21.16% dry weight for normal brain. Values less than ± 4 are probably within the normal range.

² Infused 3 hours, till death of animal.

The marked changes in brain volume described above all occurred with the brain exposed. When infusions, hypo- or hypertonic, were carried out with the skull intact the effects on the brain volume were less marked (table 2). Evidently mechanical-hydrostatic effects in the closed system counteract osmotic effects. Reid (13) observed less marked histologic effects with cats following water infusion with the skulls intact. Weed and McKibben (12), however, found histological changes following hypo- and hypertonic injections only when the skull was intact.

It may be mentioned that the amount of 25 per cent glucose solution administered during 60 to 75 minutes would correspond to 2.5 to 4.0 liters to a 70-kg. man. Yet not one of the animals so treated, with the brain exposed or the skull intact, showed any obvious signs of distress. Diuresis was prolific, but there was no hemo-

concentration; usually there was slight hemodilution as judged by hematocrit and hemoglobin determinations before and after infusion. Only the animal treated for 3 hours died. With hypotonic infusion there was no diuresis and several of the animals died.

Histological observations were made by Dr. Karl Stern. After hypotonic infusion there was swelling of many cortical nerve cells and enlargement of intercellular and perivascular spaces. The nerve cell change was most characteristic in silver stain (Bielchowsky). There was an unstained halo around the slightly enlarged nucleus and the argentophile substance was 'squeezed' in fragments to the periphery of the cell. Reid (13) found less effect on nerve cells, but constant marked swelling of oligodendroglia and no significant change in other cellular elements. The picture in areas of edema surrounding a brain tumor in man was quite different from that seen in his animals after experimental edema. The brains which had been dehydrated and shrunken by hypertonic infusion in our experiments showed, in silver stain, a peculiar nerve cell picture not unlike the one encountered in the early stages of 'senile' changes in man. The intracellular fiber strands were markedly argentophile and showed clumping and coarseness. The intercellular and perivascular spaces showed a normal picture. Details of these observations on shrunken brains are discussed in another publication (14).

These experiments and histological observations indicate that the dry-weight method does detect and roughly measure cerebral edema and dehydration. Edema from causes other than infusion of hypotonic fluid can apparently also be detected. In a series of 5 experiments no infusion was administered but, after unilaterally exposing the brain for periods up to two hours, muscle and scalp were sutured over the skull defect and the animals kept alive for two days. With 3 of these animals, dry-weight determinations indicated swelling of 3, 5.5 and 6.5 per cent. With two animals, in which the exposure was very brief, no swelling was measurable. In no case was there measurable swelling of the unexposed hemisphere.

In 9 experiments no fluid was infused into the venous system but the brain was exposed for about two hours during which it was either left dry or its surface was irrigated continuously with normal saline, Ringer's, hyper- or hypotonic glucose solution or plain water. The animal was then decapitated and the brain dry weight determined. Results showed no correlation with the type of irrigation fluid used and all were within the normal range. But the average of the series, 21.6 (s.d. 0.6) corresponding to a 2 per cent shrinkage, was significantly different from the normal average and suggests a slight tendency to dehydration of the brain during exposure.

Brains removed from animals which had been left with skull defects for two days, and from some of the animals infused with hypotonic fluid, showed elevated areas moulded to the outline of the skull defect. These are presumably regions of local edema probably developing as a result of interference with circulation by the pressure of the herniating brain against the skull defect. The excess of moisture in these small zones would be too small to affect measurements on the whole brain. Attempts to measure local edema by dry weight determinations on small local areas of the rabbit brain were defeated by too great variability in tissue samples from normal brains.

Other methods for chemical evaluation of cerebral edema have been tested in a preliminary way. These depended upon the possibility that the edematous process involved a change in the amount of brain water which is free to dissolve various substances present in the blood. For instance the chloride space of the brain is about 40 per cent of the total tissue volume instead of 80 per cent which would be expected if all the tissue water were free to dissolve chloride. If the extra fluid entering the tissue in edema contained the same concentration of chloride as the plasma, the chloride content of the brain should increase to a relatively greater extent than does the water content. It can be shown that an increase of 5 per cent in the fluid content of the brain should under these circumstances change the chloride space to 43 per cent, making a 7.5 per cent increase in chloride space. The thiocyanate space is only about 15 per cent and a 5 per cent increase in brain volume made up entirely of water, free to dissolve thiocyanate, would increase the thiocyanate space to 19.7 per cent, which is a relative increase of 31 per cent. With inulin, which normally enters the brain fluid only slightly, the relative increase in edema might be very high. All these possibilities have been tested by determinations of the substances in question in the plasma and in the brain, correcting the amount in the brain for the portion accounted for by blood remaining in the brain. Sodium thiocyanate was administered intravenously or potassium thiocyanate by stomach tube at least 2 hours or 8 hours, respectively, before killing the animal; inulin was given intravenously about 35 minutes before sacrificing the animal. The chloride space of normal brains was found to be reasonably constant, values of 37.1 to 39.6 being obtained, the thiocyanate space seemed to vary widely and no inulin at all appeared to enter the normal brain. All these methods involved accurate determinations of the materials in the blood and in the brain and of the blood content of the brain. The thiocyanate and inulin methods involved interference with the animal, while the chloride method offered little increase in sensitivity. These methods were therefore not pursued when the simple dry-weight method was found to be reasonably satisfactory. But this type of experiment might give valuable information concerning the mechanism of the development of edema.

BRAIN SWELLING WITHOUT TRUE EDEMA

A rapidly developing swelling of the brain, with tendency to herniation through the skull opening, is an occasional, but disconcerting, experience of anyone who has done extensive surgery of the brain in man or in experimental animals. Pilcher (4) observed marked bulging of the brain in one out of 6 dogs following exposure of the cortex without trauma, and in 4 out of 5 when trauma to the head preceded exposure. He was unable to show, however, that this was accompanied by a significant increase in water content of the brain in these animals. He concluded (5), "It seems probable that other factors, such as cerebrospinal fluid volume and intracranial blood volume are of greater importance than cerebral edema in producing the increased intracranial pressure which follows trauma to the head."

Prados *et al.* (15) in studies on the effects of exposure on cat brains, reported swelling usually observed about two hours after exposure. "The degree of swelling varied a great deal from one experiment to the other, and it depended on some factor the nature of which we are not yet able to determine." In our experiments on rabbits, marked herniation of the brain occurred in 4 out of 18 cases after simple exposure of the cortex for an hour or more without obvious trauma. We were unable to determine the conditions which provoked this swelling, since it did not seem related to the type of irrigating fluid being used, nor did it depend upon whether the cortical surface was kept moist or allowed to dry. The herniation subsided upon severing the neck of the animal. Brain dry-weight determinations in two such cases where herniation had occurred indicated that no swelling of the tissue due to increased fluid content had occurred.

These observations serve to emphasize again the importance of a type of swelling which can occur independent of actual change in brain tissue volume. It presumably results from blood vessel dilatation or dilatation of ventricles and cisterns. The mechanism is one of 'inflation' rather than edema. It is commonly seen in very acute form when the animal struggles and cerebral vessels become engorged. It can be readily imitated by increasing the blood volume by rapid intravenous injections or by increasing the spinal fluid volume by intracisternal injections. It may result from changes in blood volume or pressure or from increased spinal fluid volume produced by increased rate of secretion, decreased rate of absorption, or displacement of fluid from the spinal canal into the ventricles. Such events may occur as a reaction to chemical products of trauma or as a result of nervous reflex reaction to certain cerebral stimuli. Obrador and Pi-Suner (16) have described sudden inflation of exposed dog brain on the production of lesions near the fourth ventricle. The mechanism should be susceptible to partial analysis by determinations of blood in the cranium following sudden constriction of the neck by the method of White *et al.* (2). Results of some preliminary trials did not indicate that excess blood could account for swelling and herniation observed.

The observations of inflation of the brain have all been made upon exposed brains. When the skull is intact, factors which tend to produce inflation may still operate and it seems probable that some cases of raised intracranial pressure may be due partly to these factors and not entirely to true tissue edema or space-occupying lesion.

In the experiments described earlier, on infusion of hypotonic fluid into rabbits with exposed brains and on rabbits which had been kept for two days with a large skull defect, marked herniation of the brain occurred. This herniation usually subsided considerably on decapitation even though dry-weight determinations showed that there was an appreciable true increase in brain volume. In these animals, therefore, both true edema and inflation were induced.

SUMMARY

The approximate extent of swelling or shrinkage of brains of experimental animals can be readily calculated from the dry weight of the brain without knowledge of the actual brain volume. A simple method for determining the dry weight is described.

The moisture content of normal rabbit brain varies considerably. Changes in moisture content well beyond the range of normal variability were produced by intravenous infusions of hypo- or hypertonic solutions. Such changes were more marked when the brain was exposed than when the skull was intact. The difference between true edema, or swelling due to excess water in the brain, and an apparent increase in volume due to hydrostatic effects is discussed.

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PULMONARY LESIONS IN GUINEA PIGS WITH INCREASED INTRACRANIAL PRESSURE, AND THE EFFECT OF BILATERAL CERVICAL VAGOTOMY¹

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EDEMA and congestion of the lungs are frequently encountered at necropsy in head injury cases. Moutier (1) noted the frequent occurrence of fatal acute pulmonary edema within 24 hours after local cerebral trauma associated with battle injury. He attributed these deaths to pathological hyperactivity of the suprarenal glands. Antonini and Biancalani (2) reported lung edema in 41 per cent of 82 cases of head injury surviving from several minutes to several days. Astuni (3) presented a similar series of 197 autopsied cases in which 29 per cent displayed pulmonary edema. Weisman (4) collected data on the weights of the lungs in 686 cases of traumatic and spontaneous intracranial hemorrhage. In approximately two-thirds of the cases the combined weight of the lungs was greater than 900 gm., while in a control group only 2 per cent showed lungs of this size. The increased weight was due chiefly to edema and congestion, and partly to pneumonia. Pulmonary edema and congestion developed in most cases of fatal intracranial hemorrhage within 30 minutes to one hour after the injury.

Several investigators have studied experimental pulmonary edema following nervous system damage. In 1874, Nothnagel (5) reported the death of a rabbit from pulmonary congestion after probing the brain at unspecified points. Benassi (6) introduced fluid into the cranium through a trephine opening in order to obtain hypertensive coma in rabbits and dogs. In some (numbers not stated) cases, rales were heard on auscultation of the lungs, but frothy fluid was not observed in the respiratory tree. Post-mortem examination of the lungs revealed subpleural ecchymoses, emphysema, intense vascular congestion, and sometimes partial edema.

Farber (7, 8) produced fatal lung edema in guinea pigs with bilateral cervical vagotomy and attributed this edema to disturbances of the vasomotor control of the pulmonary vessels. He maintained the animals on artificial positive pressure respiration following tracheal cannulation, (insufflation pressure not stated). Various investigators (9, 10) have presented contradictory evidence, and recently Sussman *et al.* (11) stated, "There is no evidence that vagotomy exerts an influence on the pulmonary vascular system favoring edema or hemorrhage."

Sussman found massive lung edema within six hours in either intact or vagotomized guinea pigs maintained on artificial respiration at 20 mm. Hg insufflation pressure in contrast to the absence of lung edema in such animals with an insufflation pressure of 6 mm. Hg. Luisada and Sarnoff (12) employed massive, rapid venous infusion in dogs simultaneously with vagal stimulation and concluded "electrical stimulation of either the cardiac end of the cut vagi or the intact nerve favors pulmonary edema by causing extreme bradycardia." Recently, Surtshin, Katz, and Rodbard (13) have questioned whether in previous attempts to produce pulmonary edema by elevated intracranial pressure the effects were due to genuine edema or were artefacts resulting from the aspiration of saliva.

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METHODS

Guinea pigs were used in this study of lung edema and congestion following increased intracranial pressure. The anesthetic agent employed was sodium pentobarbital, 10 to 20 mg. intraperitoneally. A drill opening $1\frac{1}{2}$ mm. in diameter was made in the mid-calvarium and a balloon-tipped plastic tube was carefully inserted into this opening. Wire hooks two millimeters proximal to the latex balloon were attached to rubber bands; the latter were anchored in an infero-lateral manner in order to immobilize the balloon. One and one-half cubic centimeters of air were introduced within 15 seconds through the plastic tube into the balloon. The elevated intracranial tension was maintained until the guinea pigs were guillotined 5 minutes later. A control series of anesthetized guinea pigs were guillotined, and both the experimental and control animals were held with the severed neck dependent until bleeding had ceased.

The body weight, the weight of the heart ventricles, and the total weight of the lungs minus trachea were recorded for each guinea pig. Lung weight/ventricle weight ratios and lung weight/body weight ratios were used as supplementary aids to gross examination of the lungs for a better quantitative analysis of pulmonary pathology.

RESULTS

The data from 24 guinea pigs, which were subjected to increased intracranial pressure for 5 minutes prior to guillotining, and 26 control animals are listed in tables 1 and 2 respectively. The experimental group in table 1 displays a lung weight/ventricle weight ratio varying from 2.13 to 5.69 with an arithmetic mean of 3.61. A range from 1.74 to 3.28 may be noted in the lung weight/ventricle weight ratios in table 2 of the control guinea pigs with an arithmetic mean of 2.41. Twelve of the 24 guinea pigs subjected to elevated intracranial tension and none of the 26 control animals revealed a lung weight/ventricle weight ratio greater than 3.40. Employing the X^2 4-fold table, these differences between the control and experimental animals have a probability of less than .05 per cent of occurring due to chance, indicating a statistically significant difference between these two groups with reference to lung weight/ventricle weight ratios.

Tables 1 and 2 also contain the data regarding lung weight/body weight ratios. The differences observed between the control and experimental groups are statistically significant, for the chance of probability alone giving such results is again less than .05 per cent.

Approximately one half of the guinea pigs subjected to increased intracranial pressure were found to have lung weight/ventricle weight and lung weight/body weight ratios higher than the largest value in the control group. This same 50 per cent of experimental animals displayed pulmonary edema, congestion, and hemorrhage on gross examination of the lungs at necropsy. Figure 1 presents a photograph of the lungs of a typical animal from the elevated intracranial pressure and the control groups respectively. Asterisks in the tables identify the lungs illustrated.

The effect of bilateral cervical vagotomy was subsequently studied. The guinea pigs were guillotined 5 minutes after injection of $1\frac{1}{2}$ cc. of air into the intracranial

TABLE 1. GUINEA PIGS SUBJECTED TO INCREASED INTRACRANIAL PRESSURE

WT. OF GUINEA PIG	LUNG/VENTRICLES	LUNG/BODY $\times 10^{-4}$	WT. OF GUINEA PIG	LUNG/VENTRICLES	LUNG/BODY $\times 10^{-4}$
gm.			gm.		
376	5.32	136	340	4.65	135
308	5.69	189	400	2.27	79
292	5.10	151	355	5.12	169
371	5.12	161	315	3.59	113
300	3.46	93	207	2.52	81
350	2.67	63	369	2.44	62
330	3.12	89	210	4.31	127
344	3.88	96	217	2.90	82
309	3.02	98	210	3.80	116
340	3.22	95	Mean...291	3.51	107
319	2.96	83			
340	2.85	81			
310	2.13	75	305	2.62	78
380	3.11	93	391	3.32	90
253*	4.03	112	280	3.68	87
Mean...328	3.71	108	234	2.65	83
			358	3.29	99
			210	3.16	94
			219	2.62	84
			214	2.33	75
			195	3.12	82
			Mean...267	2.98	86

Cervical vagi isolated, left intact

Bilateral cervical vagotomy

TABLE 2. CONTROL GUINEA PIGS

WT. OF GUINEA PIG	LUNGS/VENTRICLES	LUNG/BODY $\times 10^{-4}$	WT. OF GUINEA PIG	LUNGS/VENTRICLES	LUNG/BODY $\times 10^{-4}$
gm.			gm.		
303	2.42	71	297	2.37	71
272	1.86	78	341	2.47	79
320	2.08	68	338	2.26	70
249	2.39	76	370	2.56	73
251	2.89	80	336	2.42	67
312	2.35	70	340	2.31	60
294	1.74	74	329	2.34	69
261	2.53	90	284	3.28	77
237	2.31	77	376	2.16	58
314	2.74	83	340	2.70	80
286	2.27	65	382	2.32	68
246	2.29	63	279*	2.84	67
395	2.28	64			
313	2.59	68	Mean 310	2.41	72

balloon. The cervical vagi were isolated in each animal, and a silk ligature was loosely placed around each vagus nerve. A few seconds prior to the elevation of intracranial pressure, the nerves were divided in one group of 9 animals, while, in another 9 selected at random from the same lot, the vagus nerves were left intact.

Table 1 shows that 4 of the 9 non-vagotomized guinea pigs had lung weight/ventricle weight ratios greater than 3.70, whereas none of the 9 animals with bilateral cervical vagotomy presented so high a ratio. Application of the X^2 test indicates that the probability of such findings being due to chance alone is 2.3 per cent. There is a probability of only 0.8 per cent that the differences in lung weight/body weight ratios between the sham-operated and vagotomized animals in table 1 are functions of chance. In table 1, one sees that 5 of the 9 animals with intact vagi exposed to elevated intracranial pressure show lung weight/body weight ratios higher than 10×10^{-4} , whereas by contrast none of the 9 in the vagotomized group shows such a high lung weight/body weight ratio. It is evident that vagotomy exerted a large protec-



Fig. 1. THE LUNGS ON THE LEFT are those of a guinea pig weighing 253 gm. after exposure to increased intracranial pressure. Those on the right are from a control guinea pig weighing 279 gm. Both animals were guillotined. The animals were representative of the groups and are identified in tables 1 and 2 by asterisks.

tive effect against lung edema. There is certainly no indication that elimination of vagal innervation of lung vessels promoted edema production.

SUMMARY

Pulmonary edema, congestion and hemorrhage plus abnormally high lung weight/body weight and lung weight/ventricle weight ratios were found in one-half of 24 guinea pigs subjected to increased intracranial pressure. The latter was produced by the injection of air into a rubber balloon placed extradurally through a small opening in the mid-calvarium.

Bilateral cervical vagotomy in 9 guinea pigs a few seconds prior to similar elevation of intracranial tension resulted in a significantly lesser degree of pulmonary pathology. Furthermore, a significant lowering of lung weight/ventricle weight and

lung weight/body weight ratios was observed in the 9 vagotomized guinea pigs as compared to 9 animals from the same lot with intact vagi subjected to identical cerebral trauma. In these experiments bilateral cervical vagotomy exerted a protective effect against pulmonary edema subsequent to elevation in intracranial pressure.

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STUDIES IN THE FRACTIONATION OF LIVER: COMPOSITION OF REGENERATING LIVER AFTER PARTIAL HEPATECTOMY IN RATS¹

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THE regenerating liver of the partially hepatectomized rat provides an excellent tissue for the study of the relationship between growth and chemical composition. Changes in cell structure (1, 2), lipids (3), proteins (4), nucleic acid (5, 6) and cytochrome C (6) have been determined in the whole liver tissue after partial hepatectomy. During the course of a study involving the fractionation of liver proteins (7), three main fractions having distinctive physical and chemical properties could be separated. The present investigation is concerned with the dry weight, the nitrogen and the lipid contents and concentrations of whole liver, three liver fractions and the particulate material at frequent intervals following liver lobectomy.

METHODS

Inbred male rats of Wistar stock, 65 to 70 days old and weighing between 150 and 200 gm., were used as experimental animals. A stock diet consisting of Gaines Meal and Purina Checkers was always available. Partial hepatectomy was done according to the procedure of Higgins and Anderson (1) under pentobarbital anesthesia; approximately 70 per cent of the total liver tissue was removed. At varying intervals after operation, the animals were exsanguinated, the livers were removed, blotted free of excess blood and stored in a beaker surrounded by ice water. The fractionation of liver was begun within 30 minutes. A group of rats was killed for a given period to yield a total of about 30 to 40 gm. of liver.

The livers were ground in a small meat grinder and transferred quantitatively to a Waring Blendor with three times their weight of physiological saline and stirred at slightly above 0° for 3 minutes at about 5000 r.p.m. This suspension was transferred quantitatively into a metal beaker with five times the original liver weight of physiological saline. The suspension was adjusted to pH 7.0 with 1 M sodium carbonate and stirred slowly at 0° for 15 minutes. The volume (usually between 250–350 ml.) was accurately measured. Ten ml. of this mixture were homogenized at 0° in a small Waring Blendor at high speed and aliquots were removed for the

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analysis of nitrogen and total solids. The sodium chloride was taken into consideration in calculating the total solids which were determined after heating for 48 hours at 105° .

The fractionation was done on an aliquot of 90 ml. of the liver suspension. This was centrifuged in two 50-ml. Lusteroid tubes at 10,000 r.p.m. ($12,000 \times$ gravity) for 1 hour at 0° . The residues were combined quantitatively in a single Lusteroid tube with the aid of a portion of the supernatant. The residue (A) was obtained by centrifuging at 10,000 r.p.m. The combined supernatants were dialyzed for 16 hours in a cellophane sack against running tap water maintained at about 10° , and were adjusted to $pH\ 5.8 \pm 0.05$ with an acetate buffer (ionic strength, 0.4). The precipitate (B) which formed immediately was allowed to stand for 30 minutes at 0° and then removed by centrifuging for 15 minutes at 4500 r.p.m. at 0° . The supernatant of Fraction B was adjusted to a final ethanol concentration of 70 per cent with 95 per cent ethanol. A precipitate (C) formed immediately and after standing at 0° for one-half hour, the suspension was centrifuged at -5° at 4500 r.p.m. for 30 minutes. All fractions were quantitatively transferred to a weighing bottle with the aid of minimal amounts of distilled water.

The remaining liver suspension was centrifuged at 1000 r.p.m. for 10 minutes and the upper two thirds of the supernatant was syphoned off. This supernatant was centrifuged at 10,000 r.p.m. for one hour and the residue (Fraction M) which contained only mitochondria and microsomes was transferred to a vial.

The 4 fractions were dried to constant weight in a desiccator over P_2O_5 *in vacuo*. The fractions were kept in the cold room until most of the water was removed and the drying was continued at room temperature. The total lipid carbon and total cholesterol were determined according to methods previously described (8). Nitrogen analyses were done according to the Kjeldahl procedure, using a copper and selenium catalyst.

RESULTS

The 3 liver fractions may be characterized as follows: Fraction A is the saline-insoluble residue containing cells, nuclei, connective tissue, mitochondria, large microsomes and other insoluble material. Fraction B, obtained by adjusting the dialyzed saline extract to $pH\ 5.8$, contains appreciable amounts of nucleic acid and lipid. Fraction C is composed of the saline-soluble protein precipitated by ethanol and contains only traces of lipid and nucleic acid.

The data for the dry weights of each of the 3 fractions per liver are plotted in figure 1. The rate of regeneration for all fractions is greatest on the second, third, and fourth days after operation. The values for the sum of the 3 fractions are consistently lower than for the whole liver due to the loss of dialyzable and non-precipitable materials. The percentage distributions of Fractions A, B, and C of livers of control rats are 59, 26, and 15, respectively. Following partial hepatectomy, this ratio remains relatively constant, indicating a uniform rate of regeneration for the cellular and cytoplasmic constituents.

The nitrogen contents of the fractions of the control and regenerating livers are shown in figure 2. A pattern similar to that seen for the total solids is obtained.

The differences observed between the curves for the whole liver and the sum of the fractions may be ascribed to the nonprotein nitrogen. The percentage distributions of nitrogen of Fractions A, B, and C in the control group are 50, 31, and 19, respectively; during regeneration, this ratio remains remarkably constant despite the large increases in liver weight.

The distribution of the total lipid carbon values are shown in figure 3. During the first 3 postoperative days, the lipid carbon contents of Fraction A remain fairly uniform and increase sharply on the fourth day. Subsequently the values tend to decrease. The postoperative lipid values of Fraction B decrease slightly from the control range. Fraction C contains only traces of lipids. The average percentage distribution of lipid carbon for Fractions A, B, and C in the control group is 73, 26,

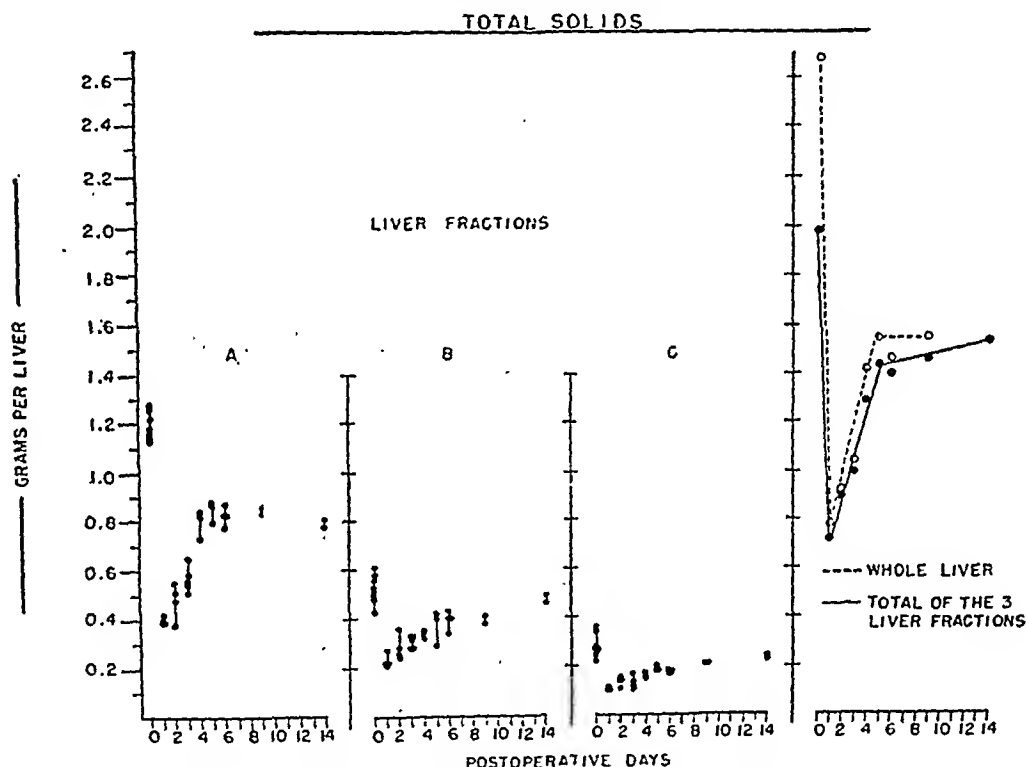


Fig. 1. TOTAL SOLID CONTENTS of 3 liver fractions after partial hepatectomy.

and 1, respectively. On the first and second postoperative days, the respective values change to 67, 31, and 2 per cent and subsequently return to the control distribution.

Cholesterol is present only in Fractions A and B (fig. 3). The cholesterol contents of Fraction A increase rapidly between the second and fifth days. The values for Fraction B decrease and remain below the control range during the experimental period. The percentage distribution of cholesterol in Fraction A and B in the control liver is 72 and 28 per cent, respectively. This ratio remains fairly constant during the first three postoperative days; during the remainder of the experiment, the ratios approximate values of 80 and 20 per cent.

Data for the percentage concentrations of total lipid carbon and cholesterol of the mitochondria are presented in figure 4. Absolute amounts are not given

since no quantitative separation of particulate matter is possible. The concentration of the total lipids is too variable for interpretation. The cholesterol concentrations show no change during the experimental period.

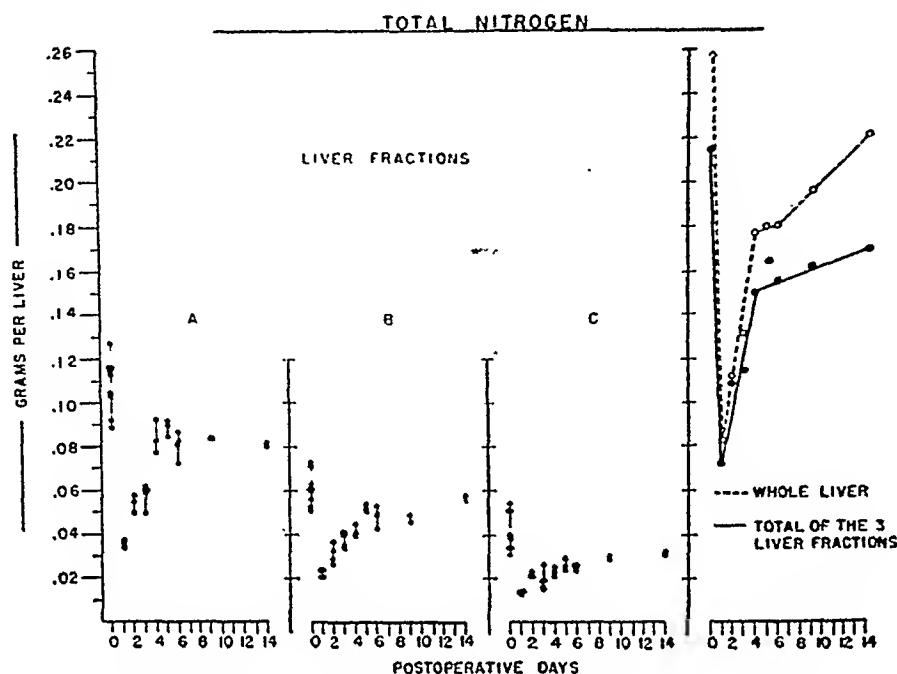


Fig. 2. NITROGEN CONTENTS of 3 liver fractions after partial hepatectomy.

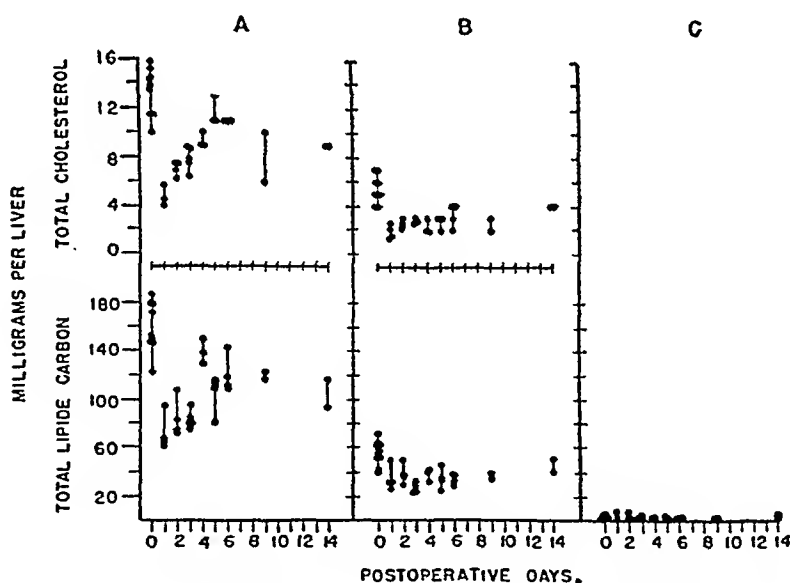


Fig. 3. LIPID CARBON AND CHOLESTEROL contents of 3 liver fractions after partial hepatectomy.

Data are presented in table 1 which summarize the percentage changes in the amounts of total solids, nitrogen and lipid carbon at varying periods after partial hepatectomy. It is seen that the changes are approximately the same for nitrogen and total solids throughout the 14-day period of observation. The changes in lipid

MITOCHONDRIA

Fig. 4. PERCENTAGE CONCENTRATIONS of lipid carbon and total cholesterol in particulate components of the liver after partial hepatectomy.

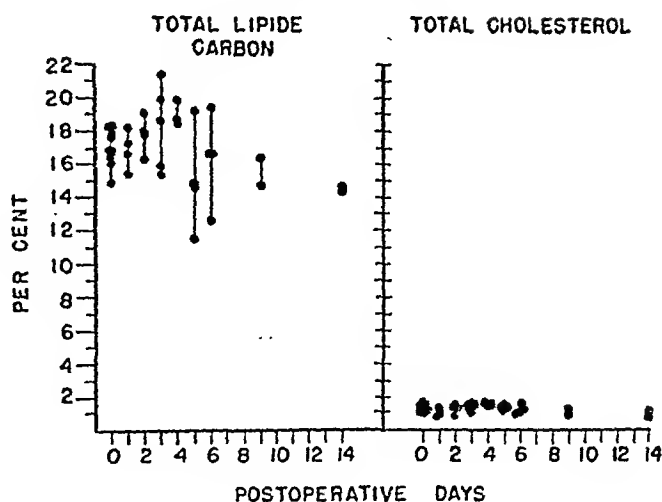


TABLE 1. PERCENTAGE CHANGES FROM THE CONTROL VALUES FOR TOTAL SOLIDS, NITROGEN, AND TOTAL LIPIDS IN THE LIVER FRACTIONS AFTER PARTIAL HEPATECTOMY

POSTOPERATIVE DAYS	FRACTIONS							
	A	B	C	A	B	C	A	B
	Total solids			NITROGEN			LIPID	
0	100			100			100	
1	34	44	35	33	35	34	46	63
2	41	54	47	50	49	56	53	70
3	58	58	46	54	58	47	53	50
4	68	65	56	78	64	56	87	70
5	73	65	65	83	79	64	66	63
6	71	75	61	75	75	61	76	63
9	73	76	73	78	73	73	74	66
14	68	93	82	75	87	78	67	82

TABLE 2. COMPARISON OF THE LIPID CARBON CONTENTS OF FRACTIONS A AND B WITH CALCULATED 'CONTROLS'

POSTOPERATIVE DAYS	FRACTION A			FRACTION B		
	I	Calc. ²	$1/2 \times 100$	I	Calc. ²	$1/2 \times 100$
	Content	'control'		Content	'control'	
	mg.	mg.		mg.	mg.	
0	164	164		56	56	
1	73	53	138	35	20	175
2	85	81	105	39	27	144
3	84	89	95	28	32	88
4	139	123	113	39	36	108
5	105	136	77	35	44	80
6	121	123	98	35	42	83
9	119	128	93	37	41	90
14	107	123	95	46	49	94

carbon are not comparable to those observed for nitrogen and total solids during the first few postoperative days.

In order to assess the significance of the differences between the nitrogen and lipid carbon changes, the lipid carbon contents are compared with calculated 'control' values (table 2). The 'control' lipid carbon data represent values for Fractions A and B of regenerating livers in which constant lipid:nitrogen ratios of control livers are maintained. The 'control' data are obtained by multiplying the nitrogen contents of each fraction by factors representing the lipid carbon per mg. of nitrogen of Fractions A (1.52 mg.) and B (0.85 mg.) of control livers. On the first postoperative day the lipid carbon contents of Fractions A and B are 20 and 15 mg. higher than the values for the respective 'controls'. The lipid content of Fraction B remains increased (144%) on the second day while that of Fraction A returns to the 'control' level. With the exception of the fourth day, at which time there is an increase above the calculated values in both fractions, all lipid carbon contents are somewhat lower than their respective 'controls'. According to these data, the increased lipid concentration of the liver during the first two days following partial hepatectomy is associated not only with the saline insoluble components but with the saline-soluble Fraction B.

SUMMARY

Procedures are given for fractionating rat liver to yield: a saline-insoluble residue (A), a precipitate obtained from the dialyzed saline extract at pH 5.8 (B), and ethanol-precipitated proteins (C). Data are presented for the changes in dry weight, nitrogen, total lipid and cholesterol contents of these 3 fractions after partial hepatectomy.

During liver regeneration following partial hepatectomy, the contents of the total solids and nitrogen of each of the 3 respective fractions are closely parallel, and do not differ in their percentage distributions. Excessive amounts of lipid are present in Fraction A and B during the first and second postoperative days. The lipid carbon and cholesterol concentrations of the particulate materials remain relatively constant during liver regeneration.

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CALORIE INTAKES IN RELATION TO THE QUANTITY AND QUALITY OF PROTEIN IN THE DIET¹

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THE relation between growth and food intake, and the relative merits of controlled and *ad libitum* food intakes in the conduct of nutrition experiments, have provoked much discussion. This has been especially true in studies of the nutritive value of proteins where the results could not be expressed in terms of units of the nutrient, and perhaps because gain in weight appears to be more directly related to protein deposition in the body than to the deposition of other nutrients. It is probably fair to say that the superiority of either method for the evaluation of proteins with young animals has not been proven but sufficient has been written so that it is unnecessary to reiterate the arguments here.

Hegsted and Worcester (1) found that the measurement of food intakes of rats receiving diets of constant protein content but of different nutritive value, and the subsequent calculation of protein efficiency (gain per gm. of protein eaten), did not yield significantly more information about the value of the protein than the simple measurement of gain alone. Gain and efficiency were found to be very closely correlated and appeared to measure the same characteristic of the diet. Protein efficiency was therefore discarded as a relatively useless concept. Since the diets used in that study were of constant protein content, the protein intake was proportional to the food or total calorie intake. Caloric efficiency, had it been calculated, would have shown the same correlation with gain as was found for protein efficiency. It is well known, however, that the percentage of protein in the diet has a pronounced effect upon the value obtained for the efficiency of any particular protein. With diets of varied protein content, the caloric and protein efficiency would not be parallel since the protein intake would not be proportional to the calorie intake but to the product of the food intake and the protein content of the diet. We have therefore studied the relation between calorie intake and growth when the nutritional value of the diet is varied either by changing the protein content or the quality of protein in the diet.

EXPERIMENTAL

Six separate experiments were made at various times during a 2-year period in which 5 different proteins were fed at several levels in the diet. These included vitamin-free casein, beef protein, and 3 different samples of soy flour which had received different degrees of heat treatment. The samples and the levels incorporated in the diets are shown in table 1. The diets contained in addition to the quantity of supplement required to supply the desired level of protein, 4 per cent corn oil, 4 per cent salt mixture (2), 1 per cent Wilson's liver extract 'L', and glucose to complete to 100 per cent. Crystalline thiamine hydrochloride, 200 μ g; pyridoxine HCl, 200 μ g; riboflavin, 400 μ g; calcium pantothenate, 1500 μ g; niacin, 2500 μ g; and cho-

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line chloride, 150 mg. were added to each 100 gm. of ration. One drop of haliver oil was administered weekly to each animal. Small groups of male rats weighing approximately 45 gm. housed in individual cages were fed each diet *ad libitum* for 4 weeks. Food intake was determined daily and the animals were weighed twice weekly. Four or 5 animals per group were used in the first 5 experiments while in the 6th, 24 rats were all fed the same diet containing 10 per cent of casein. Constant temperature animal rooms were not available but the temperature was usually between 24° and 26°C. Data on animals which died during the course of the experiment were discarded. In *experiment 4* almost half of the animals died for unknown reasons, probably unrelated to the diets they received.

RESULTS

The data have been studied in several ways. Many of these treatments appeared useless and others will be considered in a later paper. However, in considering gain in weight and calorie intakes it was soon apparent that these could not be directly related since, as discussed previously for protein (1), no account would be taken of

TABLE 1. LEVELS OF THE VARIOUS PROTEIN FED

EXPER.	PROTEIN	LEVELS OF PROTEIN FED	NO. OF RATS PER LEVEL
1	Casein ¹	6, 8, 10, 15, 20	4
2	Beef protein ²	5, 7, 9, 13, 18	4
3	Soy flour No. 1 ³	5, 10, 15, 20, 25	5
4	Soy flour No. 2 ³	5, 10, 15, 20, 25	5
5	Soy flour No. 5 ³	5, 10, 15, 20, 25	5
6	Casein ¹	10	24

¹ Vitamin-free, General Biochemicals Inc. ² Prepared from beef muscle, Wilson Laboratories. ³ Defatted soy flours, Archer-Daniels Midland Company, subjected to varying degrees of heat treatment. Soy flour no. 1 received least heat and no. 5 the most.

the calories required for maintenance. It appeared more logical to attempt to relate the caloric intake to the total body weight of the animal rather than to the gain in weight. The results of the analyses appear to support this supposition. Since daily food intakes are variable, mean food intakes over a considerable period of time were used and correlated with the mean body weight during the same period. Mean body weight was obtained by averaging the weekly weights.

Figure 1 is a scatter diagram of each experiment in which the logarithms of the mean calorie intake per day have been plotted against the logarithms of the mean body weight during the 4-week experimental period. While it is often not possible to show that the use of logarithms improves the correlation, probably because of the relatively short range over which the present data extend, they have been used because of the success of similar plots in the work on basal metabolism by Brody and others (3).

The appropriate regression lines were calculated for each experiment. The equations for these lines, shown in table 2, are of the type, $y = bx + a$, where $y =$ log calorie intake per day, $x =$ log mean weight, and b is the slope of the line. The standard error of the slope, s_b , and the standard error of the estimate, s_{xy} , are also shown. The lines for soy flour number 1 and soy flour number 2 have slopes some-

what, but not significantly, greater than the other 3 lines. None of the slopes is significantly different from any other when tested at the 5-per cent probability level. Therefore, all of the data may be combined as shown in figure 2, where they have been plotted on log-log paper. The regression line for the combined data,

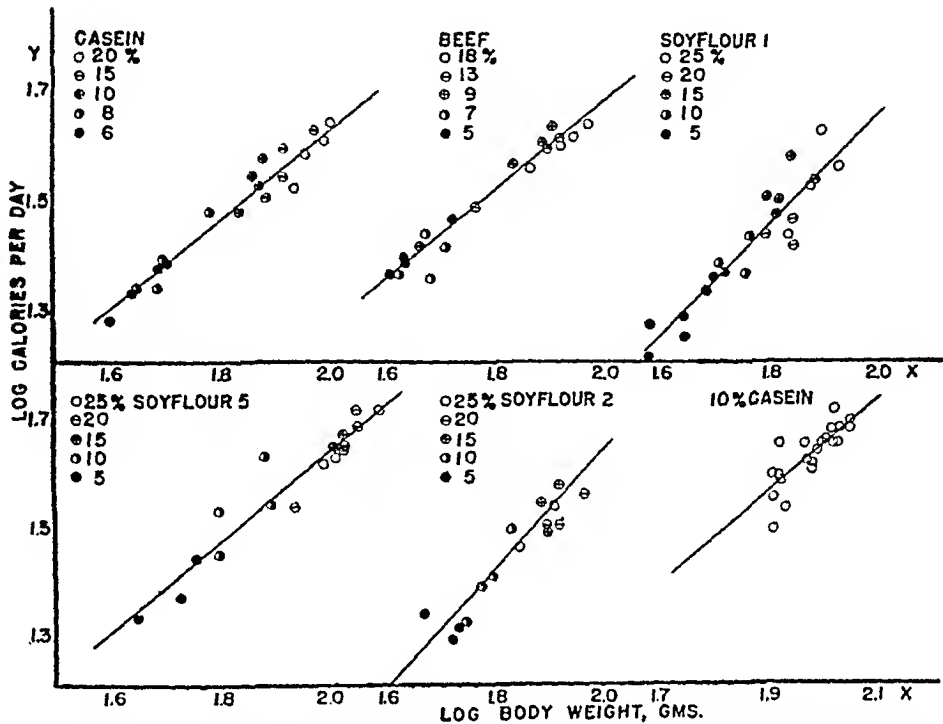


Fig. 1. SCATTER DIAGRAM showing the relation between logarithm calorie intake and logarithm body weight in the various experiments.

TABLE 2. EQUATIONS FOR THE RELATION OF LOG CALORIES PER DAY TO LOG MEAN WEIGHT FOR VARIOUS DIETS

PROTEIN FED	EQUATION OF REGRESSION LINE	r	s _b	s _{y.x}
	$Y = \log \text{ Cal.}; X = \log \text{ wt. in gm.}$			
Casein.....	$Y = .843X - .0745$.963	.017	.031
Beef.....	$Y = .823X + .0331$.976	.043	.023
Soy flour 1.....	$Y = 1.043X - .4203$.927	.094	.044
Soy flour 2.....	$Y = 1.059X - .4961$.939	.107	.035
Soy flour 5.....	$Y = .847X - .0567$.953	.069	.037
Casein (10%).....	$Y = .872X - .0956$.792	.154	.034

r = Correlation coefficient; s_b = standard error of regression coefficient; s_{y.x} = standard error of estimate.

log cal/day = .882 log wt. - .1228, becomes cal/day = .756 wt.^{.882} when the logarithms are removed. The relation of body weight to energy expenditure in resting rats and the basal metabolism of normal rats of various sizes is also shown for comparison. These data were obtained by Brody and coworkers (3, p. 406) by oxygen consumption measurements at 30°C. and, for animals of similar size, clearly parallel our data upon food consumption.

These data offered also a means of testing the effect of the length of time that the experiment is run upon the accuracy of the results obtained. The data from 2 of the experiments were analyzed separately for the first week, the first 2 weeks, etc.,

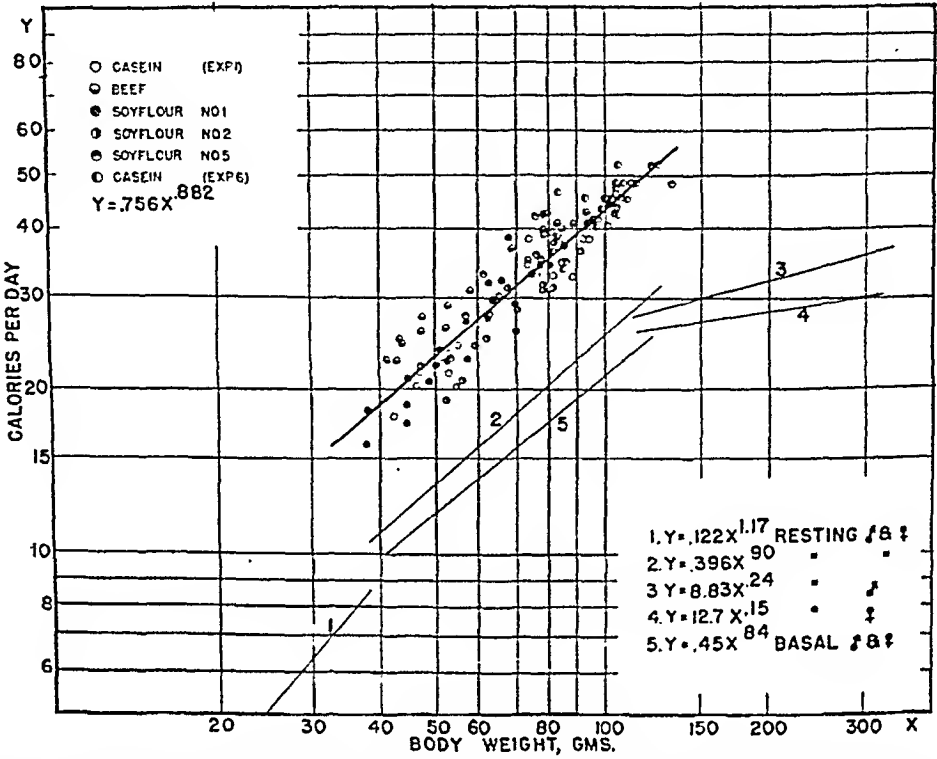


Fig. 2. COMBINED DATA FROM ALL EXPERIMENTS have been plotted to show the relation of total caloric intake to the basal and resting metabolism of rats as determined by Brody.

TABLE 3. EFFECT OF TIME ON RELATION BETWEEN LOG CALORIES PER DAY AND LOG MEAN WEIGHT AND THE ACCURACY OF MEAN GAIN

TIME PERIOD	EXPER. 1, 5 LEVELS OF CASEIN			EXPER. 6, 1 LEVEL OF CASEIN				
	wt. vs. Calories			wt. vs. Calories			Mean gain ± st. dev.	C
	r	b ± sb	sb/b, %	r	b ± sb	sb/b, %	gm.	%
wks.								
0-1				.651	.806 ± .2154	27.6	15.1 ± 5.68	37.5
0-2	.935	.891 ± .0793	8.85	.754	.824 ± .1646	20.0	37.5 ± 8.98	23.9
0-3	.957	.811 ± .0183	2.21	.802	.852 ± .1456	18.1	54.8 ± 10.10	18.6
0-4	.963	.842 ± .0176	2.09	.792	.872 ± .1539	19.4	84.8 ± 16.40	19.3
2-4	.863	.876 ± .1280	14.60					

C = Coefficient of variation of gain; r = correlation coefficient; b ± sb = regression coefficient ± standard error.

as shown in table 3. The standard error of the slope of the regression line obtained in each case has been calculated as per cent of the slope. Also, in experiment 6, where data upon a larger group of animals receiving the same diet were available, the mean gain during the various time periods, the standard deviation, and the coefficient of variation were calculated. Each criterion indicates that maximum

accuracy has been obtained after 3 weeks although the improvement over the 2-week data is only slight.

DISCUSSION

The data taken together as in figure 2 or for the individual experiments as in figure 1 indicate that, within the limits of error of the data, the mean food intake per day during the period of study was proportional to the basal metabolism or weight to approximately the 0.9 power. Since the same relation was found during the first 2, 3, or 4 weeks of the period, it would appear that the mean daily food intake at any time during the first 4 weeks was also proportional to this power of body weight. It is realized that as a matter of fact, daily food intakes are highly variable but it is convenient for the purposes of discussion and analysis to speak of daily food intakes. The constancy in the relation of food intake to basal metabolism appears to hold regardless of the rate of gain or the reason for the differences in the rate of gain, since in most of the experiments the rate of gain was controlled by the amount or kind of protein in the diet but in one experiment (*exper. 6*) the differences in gain were due to differences inherent in the animals themselves, all animals receiving the same diet.

It may be argued that the regression lines showing the relation of calorie intake to body weight in figure 1 have not been proven to be the same and this must be admitted. It can be said that the data, as they stand, do not indicate significant differences in the relation in the 6 experiments and even if minor differences due to diet be admitted, the more important fact remains that the food intakes are approximately parallel to the basal metabolism. The total calorie intake is of course considerably above the calories expended by resting rats or rats in the basal state. The difference between our calculated line and that of Brody (fig. 2) may be accounted for as difference in temperature at which the studies were done, activity in our animals, energy consumed but undigested in our animals, etc. From the data of Swift and Forbes (4) it would appear that the basal metabolisms of our animals would be approximately 20 per cent above Brody's data because of the differences in temperature. A comparison of the two lines seems valid since differences should be relative throughout the study although due to several factors just mentioned.

Assuming that the mean caloric intake is a constant percentage above the mean basal metabolism for all of the animals regardless of the rate of gain, a rough estimate of the distribution of the calories eaten may be made as shown in table 4. The computation is subject to criticism since possible differences in body composition have not been considered. However, the data of Bosshardt *et al.* (5) indicate that differences in body composition may be expected to be slight, contrary to the conclusion of Mitchell and Carmen (6). The difference in caloric intake and basal metabolism gives the calories available for growth, activity, etc. These are essentially constant when expressed as percentage of the basal calories (column 5). The gain times 2.5 cal/gm. gives the calories deposited as tissue, assuming as indicated above that the composition of the gains is not markedly different. The efficiency of the calories thus available for gain is shown in column 9. As concluded in a previous publication (1) the efficiency is very closely related to the rate of gain. Finally

the calories used neither as basal metabolism or gain must be burned or metabolized, presumably in activity (column 10). These appear to be relatively constant in terms of calories but decrease rapidly when compared to the total calories available (column 11). Thus, in the relative sense, animals which are unable to grow are wasteful of calories even when the calories required for basal metabolism are subtracted. We believe them to be more active and irritable although no measurements are available. Mitchell and Carmen (6) concluded that the calorie increment due to activity in normal rats is relatively small and this was true in the well nourished rats.

The total calorie distribution may be represented by the following equation: total calorie intake = $aWt^{.8 to .9} + 2.5 \text{ gain in gm.} + 7 \text{ calories}$. Such an equation, however, is of little value as an aid in considering the factors which govern food intake. It implies that the animals eat enough calories to meet basal needs, plus enough to meet the gain which the particular diet is capable of supporting, plus 7

TABLE 4. CALCULATED DATA FROM FIG. 2 SHOWING THE CALORIE INTAKE AND THE DISPOSITION OF CALORIES IN ANIMALS GAINING AT VARIOUS RATES

1	2	3	4	5	6	7	8	9	10	
MEAN WT.	BASAL METABOLISM	TOTAL INTAKE	CALORIE INTAKE ABOVE BM		GAIN			EFFICIENCY $\frac{8}{4} \times 100$	HEAT PROD. ABOVE BM 4-8	
			3 - 2	$\frac{3-2}{2} \times 100$	Total	Daily	Daily			
gm.	Cal/day	Cal/day	Cal/day	% of BM	gm.	gm.	Cal.	%	Cal.	% of BM
45	13.78	21.61	7.83	56.8	0	0	0	0	7.83	56.8
50	15.03	23.73	8.70	57.8	10	.35	.89	10.2	7.81	52.0
65	18.75	29.89	11.14	59.5	40	1.43	3.58	32.1	7.56	40.3
75	21.13	33.90	12.77	60.5	60	2.14	5.35	41.9	7.42	35.1
95	25.80	41.76	15.96	61.8	100	3.57	8.93	55.9	7.03	27.2

calories. We do not conceive of an animal eating in this fashion and propose as an alternative, and to us more logical, interpretation that the food intake of an animal is governed by means yet unknown at a certain percentage above its normal basal metabolism. This amount of food once eaten must be disposed of. Gain will result to the extent that the protein, vitamin and mineral needs are met by this amount of food. The remainder must be used, presumably as activity. If the food eaten does allow growth, the body size will increase, the basal metabolism will be increased, and the food intake on succeeding days thus increase.

Other explanations are perhaps as valid, but these suggestions may lead to some advance in the provoking question: "Why does an animal eat the amount of food that it does under various dietary conditions?" Those who have followed the arguments of *ad libitum* versus paired feeding during the years are aware that this is more than the familiar 'chicken and egg' proposition, and is often of fundamental importance in the interpretation of feeding experiments.

Whether few or many feeding studies can be interpreted upon a similar basis remains to be determined. Changes in basal metabolism as a result of the deficiency

would obviously negate the theory but such changes were not observed in a similar study with rats fed various levels of protein by Forbes *et al.* (7). Also deficiencies which directly affect appetite, such as thiamine deficiency, could not be expected to follow the trends exhibited by the protein deficient animals in this study. As Kleiber (8) has pointed out, the efficiency of energy utilization during dietary deficiency may be lowered by decreasing the food intake, increasing the basal metabolism, or increasing the calorogenic action of the food. A decision as to whether the first or last of these 3 has occurred in any particular study will require careful consideration of the baseline to which the food intake is compared. Also, to state that the calorogenic action of the food is increased implies a positive action of the food. In the present study, it is true that a larger proportion of the calories eaten has been expended as heat, but we do not interpret this as a direct action of the food or the deficiency but merely as a necessary consequence once food which does not allow growth is eaten.

The question of the baseline to which metabolic measurements and food intakes, etc. should be compared is of considerable importance. The writings of Brody (3) and Kleiber (9, 10) contain repeated warnings against the use of surface area as the baseline and these authors have thoroughly discussed the reasons for this conclusion. Both of these authors find that in normal adult animals of various species the basal metabolism varies approximately as weight to the 0.7 to .75 power. Kleiber (10) appears to be inclined to utilize the same unit in various experiments utilizing animals of various species and ages, but the data for various species presented in his paper lend considerable support to the belief that this unit may not be applicable within any one species. The extensive data compiled by Brody (3, chap. 14) indicate marked changes in the relation of metabolism to body weight with age. In rats weighing from 40 to 100 gm. the basal metabolism apparently varies approximately with weight to the 0.84 to 0.9 power, as already indicated, while in adult male rats it was found to vary with weight to the 0.35 power. Evidence being accumulated in our laboratory (McPhee and Hegsted, unpublished) suggests that food intakes in adult animals likewise vary with approximately this power of body weight, $Wt^{.35}$. The recommendation of Brody and Kleiber that surface area be discarded as a unit in metabolic studies, and where ever possible the true power of body weight which corresponds to metabolism be used, should receive the recognition it deserves.

In this regard it should be noted that according to Brody, the basal metabolism in relation to body weight changes at or shortly after puberty. Studies continued for long periods may result in some of the animals reaching weights where they are no longer comparable to other animals started at the same time. Since in the present studies apparently maximum accuracy was obtained within 3 weeks, long feeding experiments would appear to offer little advantage for many problems and may actually distort the results.

Finally Kleiber (10) concluded, "two animals may be regarded as being upon the same level of food intake when the rate of intake of metabolizable energy is the same multiple or the same fraction of the standard metabolic rate." Our *ad libitum* fed animals would appear to satisfy this condition even though they were growing at

markedly different rates. If this be true, then the main argument for paired-feeding, that the food intake of animals receiving *ad libitum* feeding is not controlled, would appear to be based on false assumptions. It is clear that the food intake is controlled and, whatever the mechanism may be, these data suggest that it is controlled in proportion to metabolic rate.

SUMMARY

Twenty-five different diets in which the quantity and quality of protein were varied were fed to young male rats for a 4-week period. Analysis of the data upon food intake and body weights suggests that the mean daily calorie intake varied as the mean body weight raised to the 0.88 power. Animals receiving the same diet but which varied in rate of gain and in calorie intake because of inherent differences in the rats themselves showed a similar relation between food intake and body weight.

Published results by Brody indicate that the basal or resting metabolism of rats of this size also varies with body weight raised a power near this value (0.84-0.9). It therefore appears that the mean calorie intake was a constant percentage above the mean basal metabolic rate during the experimental period, regardless of the rate of gain or the cause of this difference in gain, i.e., inherent differences or differences in the nutritive value of the diet. It is suggested that the food intake of the animals is controlled by some means at a relatively constant per cent above the basal metabolism. If the food thus consumed contains adequate nutrients for growth, the animal grows, increases its basal metabolism, and thus its food intake upon succeeding days. If the food eaten does not allow growth, or only limited growth, the remaining calories must be consumed, probably in activity. The efficiency of the animal unable to grow is thus markedly diminished even when the calculation is made only upon the calories above those required for basal metabolism.

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EFFECTS OF ATROPINE ON FOOD INGESTION AND WATER DRINKING IN DOGS

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AN ASSOCIATION of belladonna with anorexia has been reported by Rivera Pérez (1). Montgomery (2) reported that large doses of atropine did not affect significantly the water intake of dogs with or without salivary glands. Also it was observed that there was no change in the two-hourly intake of water after injection of the drug. No change in water intake was observed with pilocarpine. In another study Montgomery (3) observed that total extirpation of the salivary glands in dogs did not result in a decreased water intake. It was concluded that it was improbable that the salivary glands were a major factor in thirst regulation. Steggerda (4) in a study of the relation of thirst to dryness of the mouth in a human subject concluded that water intake is not necessarily related to a dry mouth. Studies of salivary conditioning in atropinized dogs and of pilocarpine conditioning were made by Finch (5, 6). Also the effects of atropine, pilocarpine and other drugs on oral and pharyngeal mucous secretion were reported by Montgomery (7).

The following experiment is a study of the effects of several doses of atropine sulfate on food ingestion and water drinking in dogs.

METHODS

The experiment was divided into four periods. Six adult female dogs served as subjects. The animals were confined separately to cages. A block chow (Purina) was administered as the only source of solid food in all periods. The dogs were accustomed to this diet. Drinking water was available in the cages.

Four dogs were used in Period I. Each animal was injected intramuscularly every other day with .3 mg. atropine sulfate and on alternate days with isotonic sodium chloride solution. This routine was interrupted one day in every 7 days when the animals were not injected but had food and water available for the usual length of time. The interval of feeding was 45 minutes and began 30 minutes after the injection. The animals were fed at approximately the same time each day. The volume of water drunk during the interval of feeding was recorded. Likewise the volume of water drunk over the remainder of the 24-hour interval from a liter of water placed in the cage was recorded. The period consisted of 20 days on 10 of which the animals were injected with atropine. The data of this and subsequent periods are expressed in daily averages in table 1. The experiment was conducted in Period II identically as in Period I, except that the animal received 1 mg. of pilocarpine hydrochloride in place of atropine sulfate. The pilocarpine was used as

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an additional control on the atropine, and at the same time similar measurements were made on the effects of this drug in the particular dose used. A few averages in Period II are based on 8 and 9 days. Room temperature reading was recorded daily.

The length of Period III was 40 days. This period consisted of 8 intervals of 5 days each. Each interval was separated by 2 days. In the first 4 intervals, consisting of 20 days, each animal was injected with isotonic sodium chloride solution

TABLE I. AVERAGE VALUES OF DRY FOOD INGESTED AND WATER DRUNK

DOG	DRY FOOD INGESTED		WATER DRUNK IN FEEDING INTERVAL		WATER DRUNK DAILY	
	A	B	A	B	A	B
PERIOD I: <i>A, sodium chloride control; B, .3 mg. atropine sulfate</i>						
	gm.	gm.	ml.	ml.	ml.	ml.
1	144	72	47	4	509	294
2	171	96	78	16	569	372
3	182	98	109	.5	731	479
4 ¹	194	213	104	103	797	792
PERIOD II: <i>A, sodium chloride control; B, 1 mg. pilocarpine hydrochloride</i>						
1	176	153	51	53	623	498
2	182	209	91	94	642	751
3	192	209	99	101	837	847
4 ¹	218	210	119	88	852	916
PERIOD III: <i>A, sodium chloride control; B, .6 mg. atropine sulfate</i>						
5	128	58	63	3	458	278
6	466	267	208	25	2005	1137
2	149	122	41	13	477	497
3	228	133	127	5	956	586
PERIOD IV: <i>A, sodium chloride control; B, 1.5 mgs. atropine sulfate</i>						
1	190	4	117	15	659	56
2	202	32	95	11	678	161
3	259	20	123	11	1016	144
4 ¹	212	255	195	180	875	1047

¹ Control.

one hour before feeding time which lasted one hour at approximately the same time each day. Drinking water was available during the interval of feeding and during the remaining 23 hours. The last 4 intervals of 5 days each were conducted similarly excepting each animal was injected intramuscularly with .6 mg. atropine sulfate instead of the placebo solution of saline. The same measurements were made in Period III as in Periods I and II.

In the last phase of the experiment, Period IV, 3 of 4 dogs were injected each daily with 1.5 mg. atropine sulfate for an interval of 6 days. Then the same animals

were injected with isotonic saline for another 6 days. Otherwise the routine of this period was identical with that in Periods I and II. The fourth animal served as a control. Body weights were recorded at the beginning and end of each of these intervals.

RESULTS AND DISCUSSION

Dry Food Ingestion. The injection of the .3 mg. atropine sulfate was accompanied by a pronounced food intake inhibition in the animals. The dry-food ingestion of dogs 1, 2, and 3 in the atropine interval in Period I was 50, 56, and 54 per cent, respectively, of that in the control interval. The ingestion of the control animal at the same time was 110 per cent. The average dry-food ingestion of the identical animals in the interval when 1 mg. pilocarpine hydrochloride was injected was 87, 115, 109 and 96 per cent, respectively, of that in the control interval in Period II. It is concluded from these data that the atropine sulfate exerted a real food ingestion inhibitory effect. The pilocarpine hydrochloride seemed to have no significant effect on the quantity of food eaten. The average daily room temperature in the control interval in Period I was 21°C., while that in the atropine interval was 19°C. The average room temperature in each of the intervals in Period II was 20°C.

The dry-food ingestion of dogs 5, 6, 2, and 3 in Period III when .6 mg. atropine sulfate was injected was 45, 57, 82 and 58 per cent, respectively, of that in the control interval. The decrease in dog 2 was not as marked as in Period I. Again here it seems there was a definite inhibitory effect of the atropine on food ingestion. The average daily room temperature in the control interval in Period III was 21°C., while that in the atropine interval was 17°C. The lowered room temperature in the atropine interval resulted probably in the ingestion of more food by the animal than if the temperature were higher.

The most marked inhibition of food intake occurred in Period IV when the dogs were injected with 1.5 mg. atropine sulfate. Dogs 1, 2, and 3 ingested only about 2, 15 and 8 per cent, respectively, of the dry food ingested in the control interval. The control animal ingested about 120 per cent. The average room temperature in the sodium-chloride interval was 23°C., while that in the atropine interval was 19°C. The marked decrease in dry-food ingestion after the drug was administered occurred in spite of the drop in average room temperature.

It was observed in Period IV that the atropine was still very effective on the sixth day of administration. However, release after cessation of the drug was very prompt. The animals regained their controlled eating patterns practically at once. In the 6 days of atropine administration in Period IV dogs 1, 2, and 3 lost about .9, .6, and .7 kg., respectively. The control animal lost about .3 kg. in the same time. The former three animals regained about .5, .3, and .5 kg., respectively, in the control interval. The control animal's weight increased about .1 kg.

The food intake inhibiting effect of atropine is evident in these experiments. The question rises as to how the atropine produces this inhibition. Perhaps the mechanism of inhibition may operate at some central nervous system level. Such a level has been suggested for the mechanism of d-Amphetamine-induced decrease in appetite (8, 9).

Water Drunk. The effects of the atropine on the volume of water drunk in the feeding interval and that drunk daily in Period I can be deduced from the data in table 1. There was a marked inhibition of drinking in the feeding interval after the drug had been injected. These data seem to be somewhat in disagreement with those of Montgomery (2) who reported that there was no change in the 2-hourly intake of water after injection of atropine. Conditions were not exactly the same in both studies, however. In the present experiments the measurement of water intake after injection was made during the only time when food was available, as described earlier.

The water drunk daily by the atropinized animal was much less than that of the same animal in the control interval, as shown in table 1. Yet roughly the ratio of water drunk daily to dry food ingested in the control interval was disturbed but little by the administration of the atropine or pilocarpine. On this basis, it is assumed that total daily drinking was changed little by either drug. However there was some indication that the atropine may have stimulated the drinking of water slightly over the 24 hours. The same observations were made in Period III and IV with respect to the effects of the atropine sulfate on water drunk in the interval of feeding and daily.

The question rises as to what extent the urge to drink may be modified by salivation. It may seem that the probable decrease in salivation in the animal as a result of injection of the atropine might have resulted in increased drinking of water. However, there was no evidence of this at least several hours after administration of the atropine. In fact, there was an inhibition temporarily. These results and the work of others (2-4) indicate that the salivary glands may not be the primary regulator of water intake. Perhaps the mechanism of thirst operates at some central nervous system level.

SUMMARY

Dogs were injected with atropine sulfate and the effects of the drug in the doses used were observed on ingestion of food and drinking of water. The atropine exerted a real food ingestion inhibitory effect. Also there was generally an inhibition of water drinking immediately after injection of the atropine as explained, although there may have been a slight stimulation of water drinking over the 24 hours. The mechanism of the inhibition of food intake and temporary drinking of water by the atropine is not apparent. The pilocarpine in the quantity used produced no significant changes.

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ESTIMATION OF RELATIVE VELOCITIES OF PLASMA AND RED CELLS IN THE CIRCULATION OF MAN¹

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FÄHREUS (1) on the basis of variations observed in the concentration of cells and plasma in blood flowing through glass tubes of varying diameter, concluded that the velocity of the cells must exceed that of the plasma. This hypothesis received confirmation from *in vivo* experiments conducted by Dow, Hahn and Hamilton (2), who injected red cells tagged with radioactive iron, suspended in plasma stained with Evans blue dye (T-1824), into the superior vena cava of a dog; determination of the relative radioactivity of, and plasma dye concentrations in, aliquot specimens of blood drained from the left heart indicated that the erythrocytes moved slightly faster than the plasma.

The present communication describes a method for the separate estimation of cell and plasma velocity through an isolated segment of the peripheral circulation in man. Included are the results of 6 experiments, demonstrating that erythrocytes move at a more rapid rate than plasma.

METHODS AND RESULTS

By injecting instantaneously a mixture of traceable red cells and plasma at one point in the circulation and collecting samples at frequent intervals from another the relative velocities of the cells and plasma were determined in their transit past the point of sampling. In order to recover the donor cells and plasma in concentrations sufficiently high to permit accurate measurement of small samples a single capillary bed, that of the forearm, was selected for study. The injections were made into the brachial artery and the collections were taken from an antecubital vein, the circulation to the hand being excluded meanwhile by a pneumatic cuff about the wrist inflated to pressures above systolic.

The donor cells were selected for their failure to agglutinate when tested with anti A, B or M grouping serum, the recipient cells, on the other hand, being practically completely agglutinated by the test serum. Recipients were selected as test subjects only if less than 0.4 per cent of their red cells failed to agglutinate after exposure to the test serum employed. By contrast a sufficient quantity of donor cells was injected to reach peak concentrations of 20 per cent in the collected blood samples. A description of the technique and a statistical analysis of its accuracy has been reported by Young and his co-workers (3). The error of the method was judged to be ± 2.2 per cent (S. D. ± 2) (4).

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In studying the velocity of the plasma, advantage was taken of the fact that the dye T-1824 is bound firmly and instantaneously to the plasma proteins. Three parts of a concentrated suspension of red cells in plasma were mixed in a sterile stoppered flask with one part of a 0.1 per cent solution of T-1824 in normal saline. Usually 3 cc. of this mixture was taken up in a 5-cc. syringe and injected into the brachial artery.

With the patient supine the brachial artery was punctured with an 18-gauge needle through novocainized skin. The patency of the needle was maintained either by connecting it through a Hamilton manometer to a reservoir containing 5 per cent sodium citrate or by means of a stylet and repeated flushing with heparin solution. The venous needle was 13- or 15-gauge and was directed against the stream in a large vein primarily draining deeper tissues. Its patency was maintained by the slow infusion of isotonic saline through a three-way stopcock. A short length of nylon catheter was connected to the other end of the three-way stopcock to facilitate the collection of samples in Kahn precipitin tubes. Two drops of a mixture of potassium and ammonium oxalate dissolved in saline (5) were placed in each test tube to prevent coagulation and minimize hemolysis.

After occluding the circulation to the hand the valve of the three-way stopcock was turned so that blood was allowed to run from the vein through the catheter connector into a 10-cc. test tube until approximately 5 cc. of blood had been collected. The end of the catheter connector was then moved to the first of the series of precipitin tubes and the 'blank' or reference sample was obtained. While the latter sample was being collected an assistant connected the syringe containing the mixture of donor cells and dyed plasma to the arterial needle and injected the contents of the syringe.

The injection, which was almost instantaneous, was accompanied by a verbal signal to a second assistant who began to call off second intervals from a stopwatch. The end of the catheter connector was then moved from tube to tube at 2-second intervals until the timed collection period was completed immediately following which the tubes were corked and the blood mixed with the oxalate by means of gentle inversion of the tubes. When the collection period was longer than 30 seconds more than one test tube rack was used in order to prevent clotting since the tubes in the first rack could be corked and inverted, while the samples in the second rack were being collected. After flushing the three-way stopcock and its connections a duplicate series of determinations could be carried out.

If venous blood flow was too sluggish to permit the collection of sufficiently large samples or if hemolysis occurred the samples were discarded. It was essential to place both needles well within the lumen of the respective blood vessels and to prevent the venous needle from becoming obstructed through contact with the vein wall. In several instances adequate blood flow was obtained by creating reactive hyperemia in the forearm by means of a blood pressure cuff placed high on the upper arm. After the circulation to the upper extremity had been occluded for 5 minutes the pressure in the arm cuff was released and the test was carried out during the hyperemic period.

The cell counts were performed by the selective agglutination techniques of

Ashby (6) or Landsteiner and his co-workers (7), depending on the blood type of the donor, using desiccated rabbit serum to agglutinate the recipient cells. The samples were then centrifugalized at a speed of 3000 R.P.M. for one hour, and the plasma pipetted off. The plasma samples which were heavily dyed with T-1824 were diluted by pipetting 0.2 cc. of each plasma sample into 1.0 cc. of normal saline. Using as a reference the diluted plasma sample collected immediately prior to the injection of the cell-dye mixture the density of the remaining samples were determined at a wavelength of 620 millimicrons in the Coleman Junior spectrophotometer.

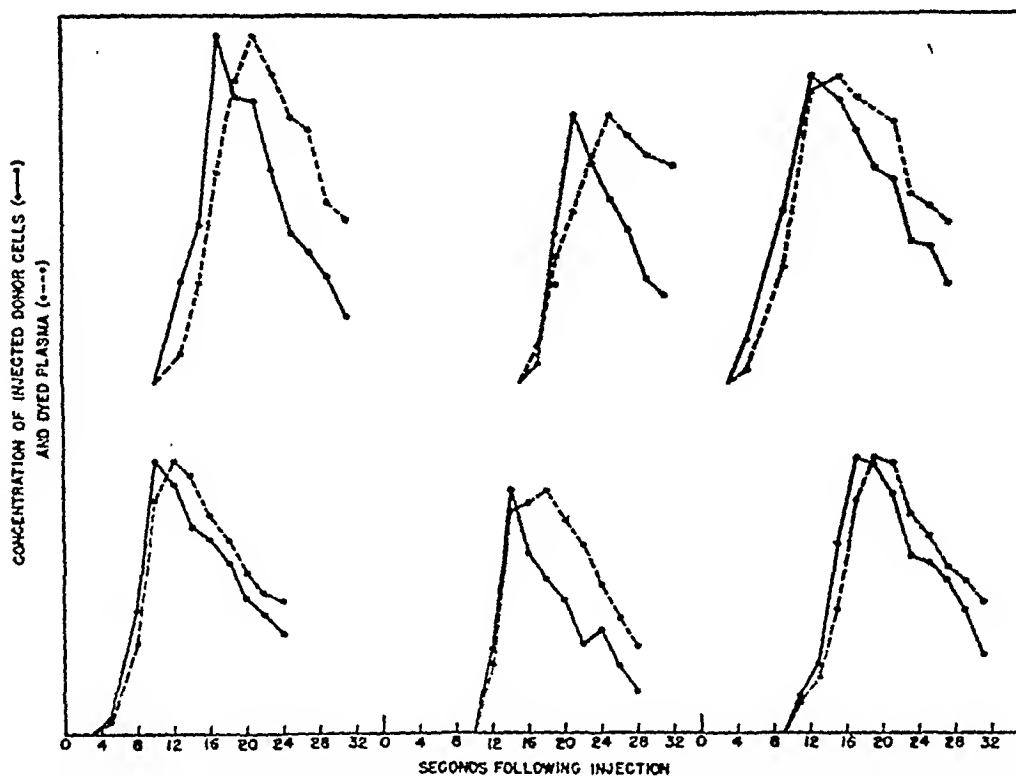


Fig. 1. TIME CONCENTRATION CURVES of donor cells and dyed plasma in 6 separate experiments on 4 subjects. The concentration scales have been adjusted in each case so that the peaks of the dye and cell concentrations fall at equal points on the ordinate.

Analysis of the curves of concentration of the injected cells and dyed plasma in six separate determinations in 4 subjects disclosed that in every case the mean velocity of the cell mass was greater than that of the plasma mass (fig. 1). Although the appearance time of donor cells and dyed plasma was simultaneous in most instances the concentration of cells had reached its peak and was already declining when the concentration of dyed plasma was still rising. In addition, the gradient of both the rising and the falling phases of the cell concentration curves usually was steeper than that of the dye curves further indicating a more rapid transit of the cell mass.

DISCUSSION

The observations that the mean velocity of the cell mass is greater than the mean velocity of the plasma mass are consistent with the laws of Poiseuille (8) concerning

the movement of fluid in tubes and of Fåhræus (1) concerning the movement of cells in the blood. The velocity of flow in the blood vessels is laminar in character because of the frictional resistance of the vessel walls and intramolecular friction, being greatest in the center of the stream and least at the periphery. This streaming effect is amplified by two factors: 1) the viscosity of the blood and 2) the relatively small diameter of the individual capillaries. Laminar flow would be most pronounced in the capillaries where a given volume of plasma is exposed to a greater frictional surface area than in the large vessels. The velocity gradient between this slow moving plasma at the capillary walls and the fast moving plasma in the center of the stream, therefore, would be large.

Further, since by Bernoulli's law (9) the lateral pressure of the fluid in a tube is inversely proportional to the velocity of flow, the larger the difference in velocity between the center and the periphery of the stream, the greater will be the gradient of lateral pressure. Hence, in the minute vessels such as the capillaries where the velocity gradient is very large, the pressure gradient from the axis to the periphery of the stream also will be large. Thus, in the capillaries the velocity will be greatest in the center of the stream but the pressure will be lowest in this region. As a consequence the cells which are in effect particles floating in a liquid menstruum will be forced into the area of least pressure, the central stream, which is also the region of greatest velocity. Thus, the greater mean velocity of the cell mass may be explained readily in terms of established laws of hydraulics.

That the appearance time was the same for the plasma and the cells may be explained by the fact that the small segment of the plasma mass occupying the central stream will traverse the capillary bed at the same speed as the fastest moving cells.

Since the characteristics of laminar flow are most pronounced in the vessels of smallest diameter it is evident that in the capillaries a considerable proportion of plasma will lose velocity through frictional resistance with the capillary walls whereas the red cells will move ahead in the central stream. Hence, as pointed out by Fåhræus (1) the proportion of cells to plasma will be less in the capillaries than in the large vessels. These data, therefore, support the conclusions of Stead and Ebert (10) and of Gibson and his co-workers (11) that the hematocrit of the capillary blood is significantly lower than the hematocrit of blood flowing in larger vessels. The falsely high hematocrit in the large vessels may explain at least in part the fact that the total blood volume calculated from the plasma volume and such a hematocrit usually is significantly higher than the total blood volume calculated from the red cell volume and plasmatocrit (11).

SUMMARY

A method is described for determining the relative velocity of identified plasma and cells during a single circulation through an isolated peripheral segment of the circulation (the forearm) in man. In 6 experiments the mean velocity of the cell mass was found to be perceptibly greater than the mean velocity of the plasma mass, thus demonstrating in the circulation of man the principles governing the velocity of particles in a stream subjected to laminar flow.

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TETRAETHYLAMMONIUM AS AN AID IN THE STUDY OF CARDIOVASCULAR REFLEXES^{1,2}

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SINCE tetraethylammonium (TEA) was shown to block transmission in autonomic ganglia (1, 2), it has been widely used as a means of assessing vasomotor tone in the human subject. Simultaneous measurements of arterial pressure and of blood flow in the extremities or in the kidneys have made possible an estimate of vasomotor tone in these vascular areas in normal and diseased subjects (3, 4); attempts have also been made to estimate the degree of vasomotor tone in hypertension in terms of the depressor response to the drug (5, 6).

Although the term 'vasomotor tone' may be interpreted to mean neurogenic constrictor tone to both arteries and veins, it is often used to signify only arteriolar vasoconstrictor tone, and therefore the neurogenic component of peripheral resistance. Except for regulation of blood flow to various organs for specific purposes (as, for example, to the skin for regulation of heat dissipation), cardiovascular reflexes are primarily adapted for the maintenance of arterial pressure. It is probable that when the so-called vasomotor center is called upon to prevent a fall of arterial pressure, all available pressor mechanisms are utilized. These mechanisms, in addition to arteriolar constriction, are cardioacceleration, probably also inotropic cardiac stimulation, and venous constriction; the three last devices, of course, can affect arterial pressure by increasing cardiac output. The integrated action of all accomplishes the desired object: maintenance of arterial pressure.

TEA should be capable of interrupting *all* autonomic influences on the cardiovascular system: cardioaccelerator and cardiodecelerator, vasoconstrictor and vasodilator, and venomotor. Although the response to this agent would, at first thought, appear to be too complex, it has nevertheless proved useful as a tool in the study of circulatory reflexes.

In the experiments described below, TEA has been used to measure reflex alterations of vasomotor tone induced by various procedures in anesthetized dogs.

METHODS

Dogs of both sexes, ranging in weight from 6 to 24.5 kg., were prepared under chloralose or thiopental-barbital anesthesia. Arterial pressure was recorded with

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a mercury manometer. Femoral and renal arterial flow were recorded in certain experiments by means of a differential manometer (7). In the femoral artery the pressure decrement across a stainless steel cannula both ends of which were inserted into the artery was measured by means of a rubber membrane manometer, each side of the manometer capsule being attached to one of the side-tubes of the cannula. For convenience in the registration of renal blood flow, the left renal artery was connected to the left common carotid artery by siliconed cannulas and plastic tubing; the resistance unit of the differential manometer was inserted in the perfusion line. Coagulation was prevented by continuous infusion of heparin. Cardiac output was measured in open-chest dogs by means of a Henderson cardiometer and a spirometer-type volume recorder. In these experiments right auricular pressure was recorded with a water manometer.

Changes in vasomotor activity were induced by carotid occlusion, vagal section, carotid denervation, carotid sinus nerve stimulation, injection of pressor and depressor drugs, hemorrhage, plethora, and asphyxia. Tetraethylammonium chloride was injected either in single doses during the sustained application of one of the above stimuli, or given by continuous infusion followed by the brief application of the various procedures. When TEA was given by injection, doses were used which produced maximal depressor responses during control periods; when given by continuous infusion, it was given at a rate double that shown to prevent completely the pressor response to carotid occlusion. In most experiments a primary dose of 5 mg/kg. followed by infusion of 12 mg/kg/hr. prevented carotid pressor reflexes; an infusion rate of 20 to 30 mg/kg/hr. was then used for subsequent procedures. Asphyxia was produced in various experiments by respiration of a mixture of 90 per cent N_2 , 5 per cent O_2 , and 5 per cent CO_2 .

RESULTS

Cardiac Output. Although TEA produces vasodilatation in the femoral bed of the anesthetized dog (1), it also exerts a positive inotropic action on the heart in sufficiently high concentration, and it was not known to what extent cardiac output changes might contribute to or modify the action of the drug on arterial pressure. To determine the relative importance of this factor, cardiac output was measured by the oncometer method in 9 experiments (table 1).

In dogs under barbiturate anesthesia, TEA produced a decrease of arterial pressure and heart rate. Maximum responses were obtained with doses of 2 to 4 mg/kg. Cardiac output was never significantly diminished in spite of the decreased heart rate. In a few cases moderate increases occurred, but in general the change of arterial pressure reflected adequately the change of peripheral resistance. Venous pressure was not altered significantly.

In animals under morphine-chloralose anesthesia the cardiac output effects of TEA were much greater. Since vagal tone is high with this anesthetic combination, the initial heart rate of such animals was lower, and TEA produced cardio-acceleration and a parallel increase of cardiac output. The increased cardiac output was accompanied by a fall of venous pressure, and was therefore considered to follow release of the heart from vagal inhibition, rather than contraction of veins or a pri-

mary increase of venous return. Arterial pressure fell, but the decrease of peripheral resistance was much greater than the change predicted by the fall of pressure alone. In all subsequent experiments the animals were anesthetized with thiopental and barbital. The arterial pressure responses to TEA may then be considered to represent chiefly changes of peripheral resistance and hence of vasomotor tone.

It is of interest that in animals under barbiturate anesthesia, heart rate decreased to 112 or less, while in animals under chloralose, heart rate increased to levels of 132 to 150 in response to TEA, although in both cases the heart must have been functionally denervated. It is more than likely that the level of circulating epinephrine is high with the morphine-chloralose combination; a single dose of TEA, by interrupting vagal impulses, would expose the heart to the circulating epinephrine, while continuous infusion, by interrupting the further secretion of epinephrine, would

TABLE 1

EXP.	CARDIAC OUTPUT			ART. PRESSURE			PERIPH. RESIST. ¹			HEART RATE		
	Before	After	%Δ	Before	After	%Δ	Before	After	%Δ	Before	After	%Δ
<i>A. Pentothal-Barbital Anesthesia</i>												
2	530	546	+3	117	97	-17	.22	.18	-18	96	80	-17
4	500	500	0	94	68	-28	.19	.14	-28	135	96	-29
6	500	490	-2	90	34	-62	.18	.07	-61	128	98	-23
7	427	470	+10	95	68	-28	.22	.14	-35	114	105	-8
9	450	446	-1	120	70	-42	.27	.16	-41	120	112	-7
<i>B. Morphine—Chloralose Anesthesia</i>												
1	540	810	+50	117	98	-16	.22	.12	-45	75	132	+76
3	607	864	+42	124	83	-33	.20	.10	-50	90	144	+60
5	520	787	+51	158	98	-38	.30	.13	-57	99	150	+51

¹ Roughly estimated as the ratio of pressure in mm. Hg to cardiac output in cc/min.

probably have resulted in a gradual decline of heart rate to levels comparable with those in barbitalized animals.

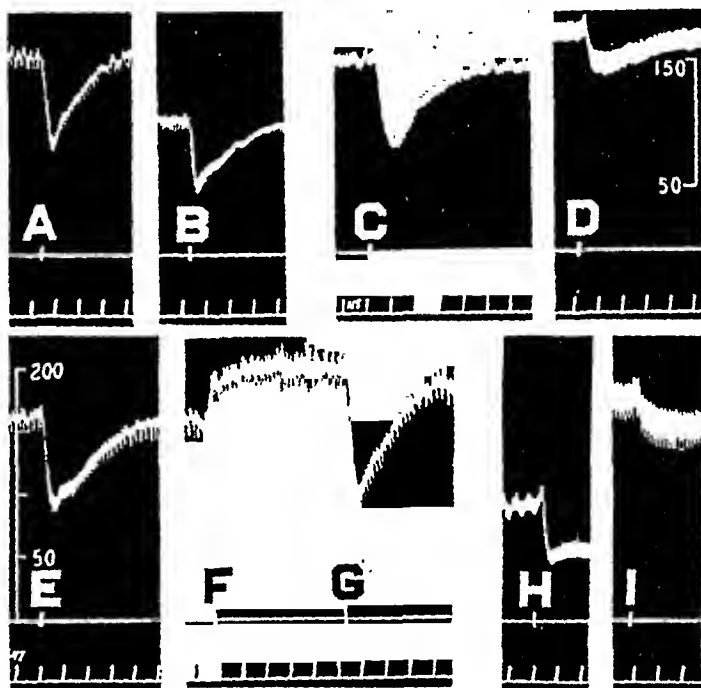
Buffer Reflexes. After determining the dose of TEA causing a maximal depressor response, both carotid arteries were clamped. Pressure increased by 20 to 90 mm. Hg in various experiments ('neurogenic' hypertension). The test dose of TEA was then repeated. In all cases, pressure fell to nearly the same floor as during control period, illustrating that the hypertension was indeed neurogenic (fig. 1, EFG). When pressure was elevated to still higher levels by carotid occlusion combined with bilateral vagal block, or by section of all the buffer nerves, TEA again lowered pressure to the original floor. The results suggested that a dose of TEA capable of completely blocking a moderate level of sympathetic outflow is also adequate to block an increased level of sympathetic tone.

To make certain that changes of cardiac output played no role in this response, the procedure was repeated in several of the cardiac output experiments. In one such experiment TEA during the control period reduced arterial pressure by 28 per

cent and total peripheral resistance by 35 per cent, with a 10 per cent increase of cardiac output (*exp.* 7, table 1). After bilateral vagotomy and carotid occlusion, the same dose of TEA lowered the arterial pressure from its elevated level by 53, and decreased peripheral resistance by 49 per cent with an increase of cardiac output of 9 per cent. Similar results were obtained in other experiments. Again the change of pressure induced by TEA reflected with acceptable accuracy the change of peripheral resistance. In contrast to the recent results of Charlier (8), carotid occlusion caused little or no alteration of cardiac output.

As expected, continuous infusion of TEA completely blocked the reflex response to carotid clamping. Depression of the response was significant at an infusion rate of 5 mg/kg/hr., and maximal at 15 mg/kg/hr. At this infusion rate, carotid occlusion raised the pressure no more than clamping of the two femoral arteries.

Fig. 1. SEGMENTS A, B, E, F, G, H, and I: dog, 24.5 kg., anesthetized with thiopental and barbital; segments C and D, dog, 16.2 kg., thiopental and barbital. A and B, responses to maximally depressor dose of TEA. (20 mg.) before and during infusion of glyceryl trinitrate, 3 gamma/kg/min. C and D, responses to TEA (40 mg.) before and during infusion of angiotonin, 0.7 units/kg/min. E and G, responses to TEA before and after occlusion (at F) of the carotid arteries. H, response to TEA after removal of 600 cc. of blood; and I, after rapid reinfusion.



Continuous infusion of TEA also prevented the cardiodecelerator and vasodepressor response to stimulation of Hering's nerve. In these experiments, maximal stimulation of the carotid nerves before administration of TEA produced moderate cardiac slowing, and reduced pressure to exactly the same level as that later produced by TEA itself, indicating that strong stimulation of the carotid receptors may completely abolish vasomotor tone.

Veratridine, which initiates reflex bradycardia, inhibition of vasoconstrictor tone, and apnea (9), failed to produce any effect upon blood pressure during infusion of TEA (10). Since both agents are capable of completely suppressing vasomotor activity, the one by ganglionic blockade and the other by reflex inhibition of the centers, depressor effects of one must obviously be prevented by the other. TEA, by blocking vagal ganglia, also prevented the reflex bradycardia induced by veratridine.

Epinephrine and Angiotonin. It has already been demonstrated that TEA does not prevent the vasoconstrictor action of epinephrine; i.e., it is not 'adrenolytic'

(1). In the present study, epinephrine and angiotonin⁵ were infused at increasing rates and the response to TEA determined at intervals.

Infusion of epinephrine at low rates (0.1–0.25 gamma/kg/min.) induced a slight fall of arterial pressure, presumably because of its vasodilator activity in low concentration. Under these circumstances TEA caused a fall of pressure to a floor which was lower than normal (fig. 2). Under the vasodilator influence of epinephrine arterial pressure had been maintained by reflex elevation of vasoconstrictor tone. Injection of TEA therefore caused a greater fall of pressure than under control conditions, and the decline in the intrinsic (floor) resistance of the vessels was unmasked by the injection of the blocking agent.

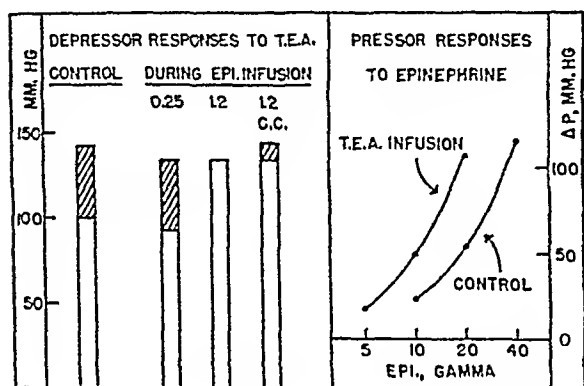
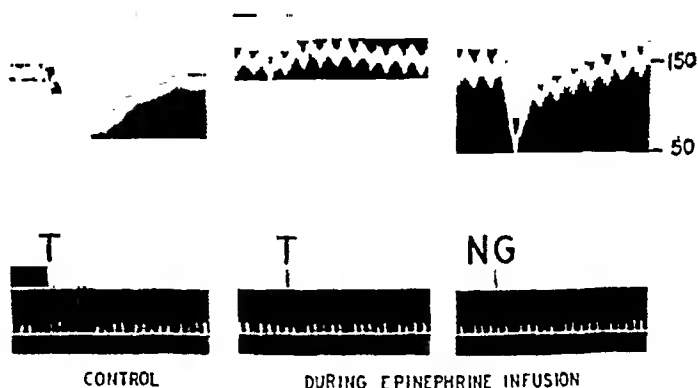


Fig. 2. (Left side) EXPERIMENT 4-13-46. Dog 9.6 kg., anesthesia, thiopental and barbital. Total height of columns, level of arterial pressure immediately before injection of maximally depressor doses of TEA; shaded portion of columns, depressor response to TEA. First column, control; second, during epinephrine infusion at a rate of 0.25 gamma/kg/min.; third and fourth columns, during infusion of epinephrine at 1.2 gamma/kg/min.; fourth column, carotids clamped. (Right side) Exp. 4-25-46, dog 24 kg. Dose response curves to epinephrine (total dose at bottom) before and during continuous infusion of TEA. (20 mg/kg/hr.).

Fig. 3. EXPERIMENT 10-21-46, dog 9.4 kg. At T, TEA, 2 mg/kg., before and during infusion of epinephrine, 0.6 gamma/kg/min. At NG, 0.05 mg/kg. of glyceryl trinitrate.



At higher infusion rates of epinephrine, although arterial pressure still did not exceed control values, the depressor response to TEA was completely lost (figures 2 and 3). It is apparent that the animal had compensated for the constrictor action of epinephrine by reflex inhibition of vasoconstrictor tone (as well as by cardiodeceleration). Compensatory mechanisms are exhausted when vasoconstrictor tone has been completely suppressed, and when this happens, TEA, of course, can cause no decrease of pressure. Frequently TEA caused a slight increase of pressure in the presence of epinephrine, for to whatever extent bradycardia was involved in the compensatory mechanism, it too was abolished by TEA (fig. 3). During infusion of

⁵ Generously supplied by the Eli Lilly & Co., Indianapolis, through the courtesy of Dr. K. G. Kohlstaedt.

epinephrine in dogs under morphine-chloralose anesthesia, in which reflex bradycardia was more prominent, TEA caused a greater increase of pressure.

During the infusion of epinephrine at rates which completely inhibited vasoconstrictor tone carotid occlusion caused a return of vasomotor activity and a further elevation of pressure; TEA again lowered the pressure to the levels existing before carotid occlusion (fig. 2). Results with angiotonin were similar, except that low infusion rates did not cause vasodilatation (fig. 1D).

Since the chief mechanism available to combat a pressure rise induced by epinephrine is reflex inhibition of vasomotor tone, it is evident that infusion of TEA will potentiate the pressor affects of small doses of epinephrine. This has already been observed (11, 12). Since the pressure rise begins from the hypotensive level established by TEA infusion, the response in mm. Hg and in percentage of the initial pressure is considerably enhanced (fig. 2); however, the peak levels of pressure reached is often not much higher than that in the absence of TEA, and even these moderate increases can be accounted for by the removal of vagal cardiac inhibition. By reflex means, sufficiently large doses of epinephrine completely abolish vasoconstrictor tone even in the absence of TEA; thus the same level of pressure will be reached as when vasomotor tone was zero initially.

Maximal potentiation of epinephrine occurred at the same infusion rates of TEA which completely prevented the pressor effects of carotid clamping.

Glyceryl Trinitrate. Infusion of this vasodilator agent activates compensatory mechanisms, chief among them being increased vasoconstrictor discharge. TEA injected during infusion of glyceryl trinitrate reduced the pressure to a floor which was lower than the control level, for the same reasons indicated above in the discussion of low infusion rates of epinephrine (fig. 1A, B). Similarly, infusion of TEA, by preventing compensatory reflexes, enhanced the depressor action of glyceryl trinitrate.

Hemorrhage and Plethora. After withdrawal of small amounts of blood, arterial pressure declined briefly and returned to control levels. Injection of TEA, by abolishing the reflexly increased vasomotor tone, now reduced the pressure to a floor lower than normal just as in the case of infusion of vasodilator agents (fig. 4). After hemorrhage, however, the lower level of pressure reached following injection of TEA must have been the result of diminished cardiac output rather than reduction of intrinsic vascular tone. Withdrawal of more blood caused further reduction of the floor level (fig. 1H and fig. 4). When hemorrhage was of moderate degree, carotid occlusion still caused a rise of pressure, though less than in control periods: vasomotor centers were therefore still not discharging at maximal capacity (fig. 4). When hemorrhage was continued to a more severe degree, the response to carotid occlusion was greatly diminished, and the full vasoconstrictor power available to the animal must have been acting in an attempt to maintain arterial pressure (cf. 13). TEA given at such a time caused a decline to very low levels, and recovery was greatly prolonged.

In two experiments blood was drawn at regular intervals until arterial pressure fell to moderately hypotensive levels, and was then reinjected. The same procedure was repeated during continuous infusion of TEA. In the presence of ganglionic

blockade each withdrawal of blood caused a greater fall of pressure, with a more prolonged and less complete recovery than during the control period.

Plethora caused by rapid infusion of heparinized blood raised arterial pressure by increasing blood volume and cardiac output. In this circumstance the depressor response to TEA was reduced (fig. 11 and fig. 4) suggesting that reflex withdrawal of vasoconstrictor tone had occurred in an effort to prevent the pressure rise. The increased floor did not, of course, represent increased intrinsic vascular tone, but rather reflected the increased cardiac output and blood volume.

Blockade of Reflexes as Illustrated by Blood Flow Studies. Injection of moderate doses of epinephrine caused an increase of mean arterial pressure accompanied by a great increase of femoral arterial blood flow. Resistance of the femoral vascular bed was reduced. During continuous infusion of TEA, epinephrine caused a greater pressor response accompanied by a relative reduction of femoral flow; resistance of the femoral bed was greatly increased (12). It is apparent that the vasodilatation

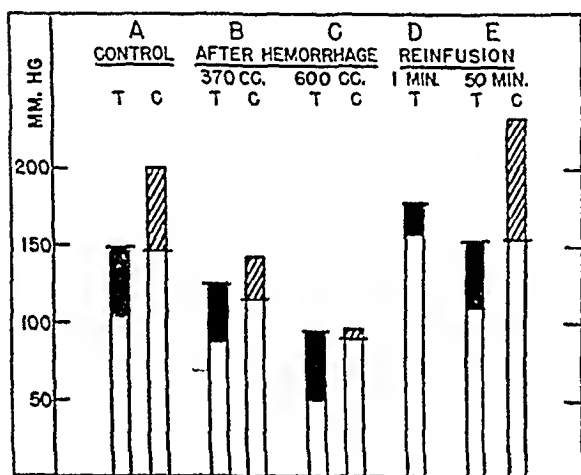


Fig. 4. EXPERIMENT 4-25-46. Depressor responses to TEA (black) and pressor responses to carotid occlusion (diagonal lines). The cross-bar on each column indicates the pressure just before each maneuver. D and E, 1 minute and 50 minutes after reinfusion of all the blood previously removed.

observed in the first instance was reflex in nature; TEA effectively prevented the reflex compensatory mechanism.

In the renal artery, blood flow was diminished by intravenous epinephrine both before and after administration of TEA. The lack of a reflex vasodilator response to epinephrine in the kidney does not imply a more intense constrictor action in the renal as opposed to the femoral circulation, but suggests that in the anesthetized dog the renal arteries are subjected to relatively little vasoconstrictor tone and therefore cannot partake in the compensatory vasodilator effort. This is confirmed by the response of renal blood flow to TEA alone. During the fall of arterial pressure induced by maximally depressor doses of TEA, renal blood flow was either unchanged or diminished, although femoral flow was considerably increased.

Accompanying the fall of pressure induced by injection of glyceryl trinitrate there was, after an initial brief increase, a diminution of femoral blood flow due to reflex vasoconstriction which persisted even after blood pressure returned to normal. During infusion of TEA, glyceryl trinitrate produced an increased femoral blood flow even though arterial pressure fell to lower levels than before (12).

Reflex vasoconstriction induced by vasodilator agents also occurred in the kidney. Injection of histamine caused a fall of arterial pressure accompanied by a decrease of renal blood flow. The diminished renal flow reflected in part the fall of perfusion pressure, but since flow recovered less rapidly than pressure, it is obvious that the renal vessels were also involved in the reflex vasoconstrictor effort. Thus it is seen that the renal vessels respond very little in compensatory mechanisms excited by pressor agents, but can partake in the compensation to depressor drugs.

Asphyxia. Although asphyxia is believed to cause intense activity of the sympathico-adrenal system, TEA prevented neither the asphyxial nor the post-asphyxial pressure rise (14). No interpretation of this anomalous response can be formulated until further studies have been completed.

DISCUSSION

These experiments indicate that in animals under barbiturate anesthesia the depressor response to TEA provides an adequate estimate of the neurogenic component of peripheral resistance. Procedures expected to increase sympathetic pressor outflow (carotid occlusion, section of buffer nerves, hemorrhage, and infusion of vasodilator drugs) enhanced the relative depressor response to TEA; procedures which reduced sympathetic outflow (plethora, infusion of epinephrine) diminished the relative depressor response to TEA. Cardiac output changes induced by the drug were slight under the conditions of these experiments. In the experiments of Eckenhoff *et al.* (15) cardiac output was significantly lower after TEA than in control observations. However, approximately an hour had elapsed between control and test procedures, and the cardiac output of control animals which had received no TEA was also diminished, confirming the report of Shore *et al.* (16) that cardiac output decreases considerably with time in animals under barbital anesthesia.

It should be recognized that under conditions of severe stress when adequate arterial pressure is maintained only by nearly maximal discharge of pressor mechanisms (hemorrhage, nitrite shock), arteriolar dilatation induced by TEA may cause a sufficient further pooling of blood to reduce venous return and hence cardiac output. Reduced cardiac output may account for occasional cases of 'circulatory collapse' reported when the drug is administered to patients with severe hypertension (17).

In human subjects measurements of cardiac output by means of the ballistocardiograph and the Fick method indicate that TEA usually causes a moderate increase, rarely a decrease, of cardiac output (5, 18). As a rule, the depressor response to TEA must underestimate the true degree of vasomotor tone. Since in the human subject in whom vagal tone is high the heart rate is increased by TEA, these results are comparable in direction if not in magnitude with those obtained in dogs under chloralose anesthesia in the present study.

The vasodepressor effects of carotid sinus nerve stimulation were completely abolished by TEA. If the depressor reflex involves excitation of vasodilator mechanisms as well as inhibition of vasoconstrictor tone (19), then it must be concluded that the vasodilator paths are also blocked by TEA. However, if vasodilator discharge is of importance in the carotid reflex, maximal stimulation of the carotid sinus nerves

should reduce arterial pressure more than does TEA itself. This was not found to occur. Furthermore, if vasodilator mechanisms take part in regulation of arterial pressure, they should be invoked as part of the compensatory effort aroused by infusion of epinephrine. That is, reflex inhibition of vasoconstrictor tone and reflex increase of vasodilator tone should *both* occur. If this be true, TEA should produce an increase of arterial pressure of considerable magnitude during infusion of epinephrine. The observed pressor responses to TEA were slight, and could be adequately accounted for by cardioacceleration (blockade of reflex vagal effects). The failure of TEA to provoke more than a slight elevation of pressure during infusion of epinephrine indicates either that activation of vasodilator mechanisms does not occur as part of the compensatory effort, or that whatever vasodilator paths are involved cannot be blocked by TEA (as would be true if such paths were non-ganglionated). It has frequently been suggested that vasodilator pathways leave the spinal cord through the dorsal roots. Such unique channels might not be interrupted by ganglionic blocking agents. However, Bernthal *et al.* (20) have demonstrated that extensive sympathectomy completely prevents carotid body vasomotor reflexes. Their evidence supports the conclusion that barostatic reflexes are mediated chiefly if not entirely by modulation of vasoconstrictor activity (including, of course, venomotor mechanisms).

Reactions of the renal and femoral vascular areas to epinephrine, vasodilator drugs, and TEA show, at least in the dog under barbitol anesthesia, that vasomotor tone is relatively low in the kidneys, and high in somatic areas. Compensatory vasodilatation in response to pressor agents can occur only in areas which are initially subject to vasoconstrictor tone; i.e., femoral and presumably other somatic vessels. Compensatory vasoconstriction can and doubtless does involve both somatic and visceral vascular beds. The evidence available shows that human buffer mechanisms may to some extent resemble those in the dog. TEA causes little change of renal blood flow, but usually a well-marked increase of flow in the hands and feet. Glyceryl trinitrate and other agents which relax smooth muscle may lower pressure, but rarely increase blood flow in the extremities; the direct action of the drug is overcome by reflex constriction. For this reason only agents which block vasoconstrictor pathways at some point may be expected to cause useful hyperemia of the extremities.

SUMMARY

The depressor action of TEA in barbitolized dogs is due to reduction of peripheral resistance. In dogs under chloralose the reduction of peripheral resistance may be masked in part by increased cardiac output. The response to TEA may be used to estimate the degree of vasoconstrictor tone existing during various experimental conditions; for example, during neurogenic hypertension, infusion of pressor or depressor drugs, hemorrhage, and plethora. By interrupting compensatory reflexes, TEA potentiates pressor and depressor drugs. By interrupting the efferent pathways, TEA prevents the cardiovascular effects of veratridine. Injection of histamine induces compensatory vasoconstriction in both the femoral and renal vascular beds; epinephrine induces compensatory vasodilatation in the femoral but not the renal

circulation. The compensatory effort induced by injection of pressor drugs appears to be accomplished mainly by inhibition of vasoconstrictor discharge; increased vasodilator activity could not be demonstrated.

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HEMODYNAMICS OF AORTIC OCCLUSION¹

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AS EARLY as 1886, de Jager (1) found that occlusion of the aorta directly above the diaphragm causes a considerable increase in the carotid pressure. Later, other authors observed blood pressure rises after clamping of large arteries and, more recently, blood pressure changes after aortic clamping were studied by Barcroft (2), by Brotchner (3) and by Hamilton and Remington (4). Most of the authors mentioned recognized the blood pressure rise as a hemodynamic phenomenon which is independent of any of the neural (1-3) or humoral (3) mechanisms which regulate the blood pressure. Barcroft made the surprising observation that occlusion of the aorta results in an increase of the cardiac output. In the present study some of these experiments have been repeated and expanded, and an attempt has been made to devise a model of the circulation which accounts for the mechanisms producing the phenomena observed.

METHODS

Since a study of hemodynamic phenomena was contemplated, all experiments were carried out on vagotomized animals in deep Nembutal narcosis, in order to depress the circulatory reflexes as much as possible without reducing the circulation to a shock level. Blood pressure was recorded with a mercury manometer connected with one of the carotids. The aorta was occluded by a loop of thick cotton string placed around this vessel between the diaphragm and the celiac artery. In some experiments the same procedure was used for the occlusion of aortic branches. Cats were used in all experiments.

RESULTS

Figure 1, I shows the blood pressure changes in a deeply narcotized and vagotomized animal when the aorta is clamped directly under the diaphragm. The general course of the pressure rise is asymptotic. The presence of the neural regulating mechanisms of heart and vessels can disturb this course as shown in figure 1, II. In this preparation the vagus nerves were not severed. The heart rate was 164 before clamping the aorta. Due to the depressor and carotid sinus reflexes the rate dropped during the pressure rise to 148, to increase again after release of the clamp to 169. The record shows that the blood pressure, after reaching a maximum,

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started to fall even though the clamp on the aorta was left in place. After release of the clamp the pressure dropped considerably below the original level to recover only slowly. In preparations in which narcosis was light these features were even more pronounced. Indications of these deviations from the asymptotic curve are occasionally seen in narcotized and vagotomized preparations and are probably due to residuals of the regulatory activity transmitted through the undisturbed part of the autonomic system. In preparations not showing this activity, the increased blood pressure is maintained during occlusion of the aorta for 5 to 10 minutes; when the clamp is then removed, the pressure drops asymptotically. The occlusion of smaller arteries has a similar but smaller effect on the blood pressure as clamping the aorta. This effect is cumulative as is shown in figure 1, III in which successively the celiac and the superior mesenteric arteries were clamped.

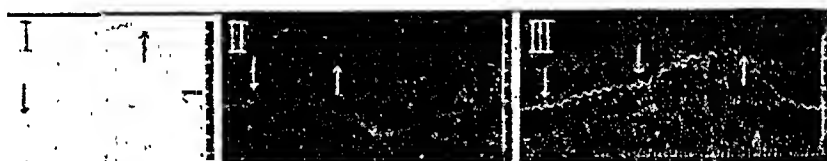


Fig. 1. EFFECT OF AORTIC OCCLUSION in a vagotomized and deeply narcotized preparation (I). The same in a preparation with intact vagi (II). Occlusion, first of the celiac then of the superior mesenteric artery, followed by a release of both arteries together (III). In this and in fig. 2 and 3 an arrow pointing down indicates occlusion, one pointing up release of the vessel. Calibration is from 9 to 16 cm. in I, from 7 to 14 cm. in II and from 8 to 14 cm. in III. Time in sec.

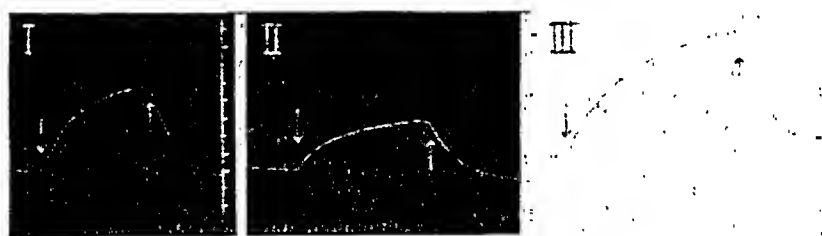


Fig. 2. EFFECT OF POSITION of the preparation on the blood pressure reaction due to aortic occlusion. I, the animal is in the horizontal position; II, tilted with the head up, and III, tilted with the head down. Calibration from 8 to 18 cm. Time in sec.

In support of de Jager's, Barcroft's, and Brochner's concept of the effect of arterial occlusion as a hemodynamic, rather than a reflex phenomenon, it was found that destruction of the spinal cord, or asphyxiation for 20 minutes of the entire central nervous system by raising the intradural pressure above the arterial pressure (5), did not abolish the reaction on aortic occlusion, even though these procedures reduced the blood pressure to a few cm. Hg. Also the injection of nicotine in doses large enough to stop conduction in the sympathetic ganglia (100-200 mg/kg. body-weight) had little effect on this phenomenon.

De Jager first came to the conclusion that after clamping the aorta blood flows cephalic from the occluded area. The importance of this flow for the blood pressure rise after clamping can be demonstrated in the following way. Tilting the animal with the head down will facilitate the flow of blood out of the inferior vena cava region, tilting with the head up will hamper that transport. Figure 2 shows that

the blood pressure response on clamping the aorta is indeed modified by tilting the animal. The response with the head up is smaller, with the head down larger than the response in the level animal. Another way of showing the importance of the transport out of the inferior vena cava region for the blood pressure response is shown in figure 3. The first pressure changes in this figure (I and II) are the usual effects of clamping and releasing the aorta. At III (fig. 3) the vena cava is occluded directly under the diaphragm. This causes a drop in blood pressure which is due, as recognized by de Jager, to a loss of blood through the aorta into the lower part of the animal. A few seconds later (IV) the aorta is clamped causing only a small rise of the blood pressure. After release of the vena cava (V), however, a blood pressure rise develops which resembles the increase of figure 3, I in which the aorta was

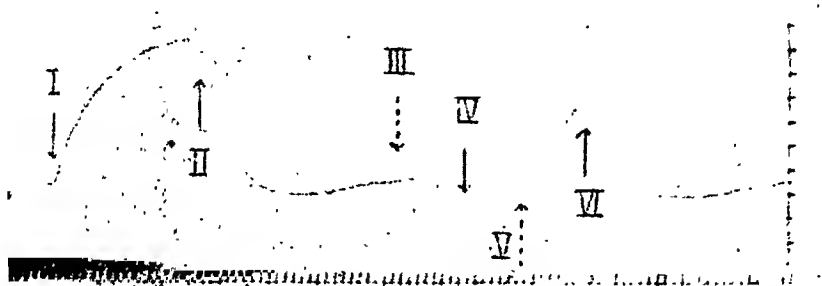


Fig. 3. EFFECTS OF CLAMPING THE AORTA and inferior vena cava. I and II, aortic occlusion and release; III, clamping of the vena cava; IV, aortic occlusion; V, release of the vena cava; VI, release of the aorta. Calibration from 7 to 16 cm. Time in seconds.

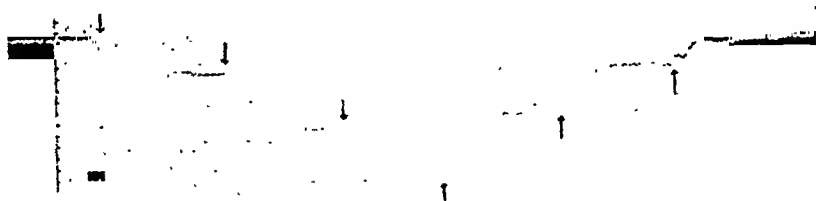


Fig. 4. EFFECT OF REMOVAL and injection of blood. At each of the arrows pointing down, 10 cc. blood is removed, at each of the arrows pointing up 10 cc. blood is reinjected. Calibration from 3 to 17 cm. The block indicates 10 seconds.

clamped while the vena cava was patent. The force which moves the blood out of the arteries and veins of the occluded area is the elasticity of the vessels. As long as the blood flow is unimpaired the vessels are extended by the pressures which cause this flow. After clamping the aorta the pressures in the occluded area become equal to that in the right atrium, causing a retraction of the vessels, which force their contents into the cephalic part of the circulation.

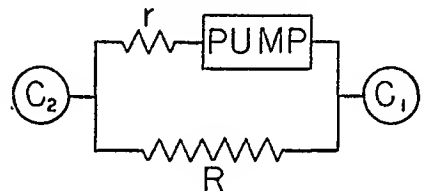
The filling of the circulatory system was found to affect the blood pressure markedly in deeply narcotized and vagotomized preparations. Figure 4 shows an experiment in which 10 cc. blood is withdrawn 3 times from the circulation in such a preparation. Each time this causes a permanent drop in the pressure. When the blood is injected again 10 cc. at the time, the pressure returns to the original level in three steps. In more reactive preparations the circulatory reflexes compensate for the effects of such relatively small changes in blood volume.

A MODEL OF THE CIRCULATION

A model including the pertinent features of the circulation is schematized in figure 5. It is assumed that the pump has that characteristic of the heart which makes the stroke volume dependent on the amount of fluid flowing into the pump between two strokes (Starling's law). The connections between the out- and inlet side of the pump consist of rigid tubing, and the elastic properties of the vascular system on the arterial and venous side are represented by the capacitors C_1 and C_2 . The peripheral resistance (arterioles and capillaries) is represented by the resistance to flow, R . The resistance r represents certain properties of the pump and also includes the resistance in the venous system.

The steady state relations among the pressures on the outlet and inlet side and the flow in the system on the one hand, and the various parameters of the circuit on the other can be found as follows. The difference between the environmental pressure and the pressure at the outlet side is designated as P_1 , the pressure between the former and the pressure in the region of the system between the resistors R and

FIG. 5. CIRCULATORY MODEL (for explanation see text)



r , as P_2 . The pressure drop for viscous flow through the resistor R , assuming that its properties are independent of pressure,⁴ will be proportional to the rate of flow, I

$$P_1 - P_2 = I R \quad (1)$$

where R includes the viscosity of the fluid.

The pump is considered as operating at constant stroke frequency, with connection alternately to the input and output sides. In filling it will operate as an elastic vessel, expanding to a volume which is proportional to the input pressure. Thus the mean rate of flow through the pump will be proportional to the input pressure⁵

$$I = P_2 / r \quad (2)$$

All additional parameters limiting the flow into the pump are lumped in the constant r , including input resistance, inertia, the time restriction of filling according

⁴ In reality the pressure-flow relation is not linear (6, 7). However in the normal pressure region the deviation from linear relationship is negligible.

⁵ It follows from the work of Patterson, Piper and Starling (8) that an increase of the heart output at constant arterial pressure is coupled with a dilatation of the heart and an increase in venous pressure, which is necessary to increase the stroke volume and also to dilate the heart. Since both of these factors causing the increase in venous pressure will be approximately proportional to flow, equation (2) will describe these conditions adequately. It follows further from Patterson, Piper and Starling's work that an increase of the arterial pressure at constant heart output is also coupled with heart dilatation and increased venous pressure. This effect of arterial on venous pressure is not represented in (2). This is probably permissible with a heart in good condition (no large systolic residue); in a failing heart, however, this simplification may become increasingly serious.

to the length of the pump cycle, etc. It will be assumed that r is constant for a given stroke frequency.

As the pressure is increased on the inlet or outlet side the capacitors will contain correspondingly larger volumes of fluid. It will be assumed that V , the increase in fluid volume in a capacitor is directly proportional to the gauge pressure,⁶ such that for the output and input capacitors

$$\begin{aligned} V_1 &= C_1 P_1 \\ V_2 &= C_2 P_2 \end{aligned} \quad (3)$$

where C_1 and C_2 are constants of capacitance for the capacitors (volume increase per unit change of pressure). For a constant total volume of fluid in the system the sum of V_1 and V_2 , which represents the volume of fluid transferable from one capacitor to the other, must also be constant

$$V_1 + V_2 = V \quad (4)$$

Eliminating I between (1) and (2)

$$(P_1 - P_2)/R = P_2/r \quad (5)$$

Substituting (3) in (4)

$$C_1 P_1 + C_2 P_2 = V \quad (6)$$

Eliminating P_2 between (5) and (6) yields for the pressure on the outlet side

$$P_1 = \frac{V(R + r)}{C_1 R + (C_1 + C_2)r} \quad (7)$$

Elimination of P_1 between (5) and (6) gives for the pressure on the inlet side

$$P_2 = \frac{Vr}{C_1 R + (C_1 + C_2)r} \quad (8)$$

From (2)

$$I = \frac{V}{C_1 R + (C_1 + C_2)r} \quad (9)$$

Some consequences are immediately obvious. Both pressures at outlet (arterial) and inlet (venous) side as well as flow (minute volume of the heart) increase with the volume of circulating fluid. An increase in peripheral resistance (R) reduces pressure on the inlet side and flow but increases the pressure on the outlet side. However, since R is present in the numerator as well as in the denominator of (7), whereas it is present only in the denominator of (8) and (9), it has to be expected that the relative effect of a change in R is considerably less on the pressure at the outlet side than on the flow and on the pressure at the inlet side. This is because an increase in peripheral resistance depresses the flow in the system and thus counteracts the effect of the higher resistance on the pressure at the outlet side. Increase of C_1 and C_2 individually or together will result in a decrease of the pressure both at

⁶ We are not aware of studies in which the filling of a vascular area is related to the pressures prevailing in its parts (arteries, arterioles, capillaries and veins) under normal conditions. The pressure-volume relation in the aorta (7), however, is almost linear. In the vena cava inferior this relation deviates from linearity especially in the region of the lowest pressures.

the outlet and inlet side and of flow. Such a change would be caused by a loss of tone in the arterial and venous system of the animal.

DISCUSSION

If the equations describing the steady state relations in the model are to represent the blood circulation, there must eventually be conformity between the observed circulatory phenomena in the animal experiments and the predictions which can be made from the equations after the introduction of appropriate parameters to make them fit individual conditions.

Marked and permanent decreases and increases of the arterial pressure were observed after removal of blood from, or injection of fluid into the circulatory system of the cat. Since such changes in V are directly proportional to the arterial pressure (7) this is in agreement with the predictions made on the basis of a study of the circulatory model.

In the model the constant of capacitance is independent of the peripheral resistance (R). In the animal experiment clamping the aorta not only increases the peripheral resistance, but also eliminates part of the arterial and venous capacitors. This is obvious for the arterial capacitor but since the vena cava is in wide connection with the right atrium where the pressure is low, the venous capacitor in the caudal part of the body is functionally eliminated even if the vena cava inferior is not actually clamped. If large branches of the systemic arterial tree are clamped, it is reasonable to assume that the changes in the arterial and venous capacitances are inversely proportional to R , since the capacitors are formed by the vessels themselves and the larger the part of the vascular system which is occluded the greater its capacitance, and also the greater its conductance $\left(\frac{1}{R}\right)$. The constants of capacitance for the arterial and venous systems therefore can be written under those circumstances as $\frac{C'_1}{R}$ and $\frac{C'_2}{R}$. Substituting this in (7), (8) and (9) gives:

$$P_1 = \frac{RV(R+r)}{C'_1R + (C'_1 + C'_2)r} \quad (10)$$

$$P_2 = \frac{RVr}{C'_1R + (C'_1 + C'_2)r} \quad (11)$$

$$I = \frac{RV}{C'_1R + (C'_1 + C'_2)r} \quad (12)$$

There is evidence that after clamping the aorta the arterial and venous capacitors in the caudal part of the animal empty into the right atrium where the average pressure is near zero. The fraction of V situated in the caudal part of the preparation thus is shifted into the remaining part of the circulation and the transferable amount of blood (V) in the reduced arterial and venous capacitors is the same as that in the larger capacitors before aortic clamping. Since after establishment of the new equilibrium following aortic clamping, V remains practically unchanged and R is greatly enlarged, an increase can be expected of the arterial and venous pressure and of the blood flow in the cephalic part of the circulation. As R appears in the nu-

erator and in the denominator of (11) and (12), the effect on P_2 and I will be small as compared with the change of R . However, since the change in R due to aortic clamping is so large, an easily measurable increase in the venous pressure and in the blood flow can be predicted. In (10) R appears to the second power in the numerator and only to the first in the denominator and thus a relatively greater effect on the arterial pressure is to be expected. It seems surprising that occlusion of a large part of the circulation would increase the flow through the heart. However, this paradoxical effect of aortic clamping has been observed. Barcroft found that occlusion of the aorta directly under the diaphragm in vagotomized preparations caused regularly an increase of the heart output up to 53 per cent (the average increase in 21 experiments was 25 per cent). The same phenomenon was observed in more than half of 26 preparations in which all reflex activity was prevented by asphyxial destruction of the brain, thus showing it to be of hemodynamic origin. In the rest of these 26 preparations aortic occlusion caused either no change of the heart output or a slight decrease. It has been remarked above, that the use of equation (2) is probably only justified when the heart is in good condition. The low arterial pressure produced by destruction of the brain may well have an adverse effect on the oxygenation of heart, and this may explain the variability of the effect of aortic occlusion in such preparations.

Barcroft as well as Hamilton and Remington (4) found, that in preparations with intact vagus nerves, aortic occlusion often fails to cause an increase in heart output. This is probably due to the activity of the circulatory reflexes, since in the data of Hamilton and Remington the preparation showing the greater reduction of the heart output during occlusion, also showed the greater decrease of the heart rate in this period.

De Jager observed during aortic occlusion the expected increase in the (jugular) venous pressure.

The relation between the change in R and the change in arterial and venous capacitances due to vascular occlusion will differ for the various circulatory areas. In the splanchnic region, for instance, with its very large venous capacitor the decrease in capacitance of the venous system caused by clamping the celiac and mesenteric arteries will undoubtedly be larger than indicated by the relation $\frac{C_2'}{R}$; in the renal circulation which has only a limited capacitance the decrease in the capacitances of the arterial and venous system will probably be smaller than indicated by that relation. The increase in blood flow will therefore in the first instance be larger than indicated by (12); in the case of the renal circulation the increase in flow will be smaller than would follow from that equation, or flow might even be decreased as compared with that before clamping. In clamping a very large part of the circulation including many vascular areas (as in aortic occlusion), it is more likely that the changes in the capacitances and the change in R indeed approach inverse proportionality.

In the experiment in which the aorta was clamped while the vena cava inferior was occluded, the transport of blood from the caudal part of the animal was prevented. The amount of transferable blood (V) in the reduced circulation thus is

smaller than in the larger circulation before aortic clamping. The reduced blood volume will be roughly inversely proportional to R , if we assume that the amount of blood in a vascular area is proportional to its size and thus to its conductivity $\left(\frac{1}{R}\right)$. The transferable volume thus can be written as $\frac{V}{R}$. The equations (11), (12) and (13) then revert to (7), (8) and (9). It would follow from the latter that a rise in arterial pressure is to be expected, which is small with respect to the change in peripheral resistance and relatively larger drops in venous pressure and flow. These predicted changes in arterial pressure and flow have been observed by Barcroft (2). Also in the present experiments the pressure rise due to aortic clamping was greatly reduced by occlusion of the vena cava inferior (fig. 3). A complication not considered until now is present in the capacitors formed by the vessels of the lung circulation and also by the heart. These capacitors are small as compared with the large arterial and venous capacitors of the systemic circulation, and their neglect when considering the normal circulation seems justified. However in a reduced circulation as after occlusion of the aorta and vena cava inferior it is advisable to take their effect into account. The reduction of the heart output due to the clamping of these two vessels observed by Barcroft, may result in an emptying of the lung and heart capacitors into the reduced systemic circulation, increasing its blood volume and thus causing a larger rise in arterial pressure and a smaller decrease of venous pressure and of flow than would follow from the equations (7), (8) and (9). Barcroft believed this transfer of blood from the lung circulation and also from the heart into the reduced systemic circulation to be the sole cause of the rise in arterial pressure after simultaneous occlusion of aorta and vena cava inferior. However, it follows from (7) that even without these capacitors a small rise in arterial pressure is to be expected.

It has been possible to explain with the equations (7), (8) and (9) and a few reasonable assumptions, a number of observations on the blood circulation and it therefore seems allowable to consider these equations as a first approximation of a mathematical description of the circulation. It is by changing the values of the various parameters that the circulatory reflexes regulate the circulation. For instance, the main effect of an arteriolar contraction is an increase of the peripheral resistance, but this will also affect the capacitance of the system. Increase in tone of arteries and veins affects mainly the constants of capacitance, although the peripheral resistance also may undergo changes. Changes in heart rate will affect r . A marked slowing of the rate will increase r , as the pericardium in the intact animal limits the increase in filling compensating for the decreased heart rate (4).

From equations (7), (8) and (9) the conclusion can be drawn that the arterial and venous pressure and the blood flow are equally good indicators for changes in the blood volume filling the capacitors, and for changes in the constants of capacitance of the latter, since there exist relatively simple direct and inverse relations among these parameters. For the same reason the venous pressure and the flow are good indicators for changes in peripheral resistance. The great difference between arterial and venous pressure shows that the peripheral resistance (R) is large with respect to r (see equations 1 and 2), and it follows from (7) in which R is present

in both numerator and denominator, that under such circumstances changes in the peripheral resistance can have only a relatively small effect on the arterial pressure. Peripheral resistance therefore should be regarded as a regulator of blood flow rather than as a primary determinant of blood pressure. These conclusions make it highly unlikely that the considerable changes in systemic arterial pressure due to activity of the regulatory mechanisms of the circulation are primarily attributable to changes in the peripheral resistance as is usually assumed. Indeed, besides changes in R , changes in other parameters much more directly related to the arterial pressure are involved in vascular reactions. For instance a generalized sympathetic activity or a release of adrenalin may cause besides 1) contraction of the arterioles, mainly increasing R ; 2) contraction of the spleen, increasing V ; 3) increase of tone in the arteries and veins, decreasing the values of C_1 but probably even more of C_2 ; 4) increase of the heart rate which will usually result in a decrease of r . The first 3 of these factors tend to increase the systemic arterial pressure (γ). The changes in V , C_1 , C_2 and r tend also to increase the flow in the system but the change in R has an opposite effect. Depending on the numerical values of the parameters involved, an adrenalin injection therefore may or may not increase the total blood flow. It is of interest to note in this respect that Tigerstedt (9) observed during stimulation of the splanchnic nerve, which produces most of the changes mentioned above, an increase of the total flow in the systemic circulation.

SUMMARY

A model of the blood circulation was proposed and conclusions drawn from its mathematical description were found to be in agreement with observed effects of aortic occlusion. The relations among arterial and venous pressure and heart output on the one hand and blood volume, peripheral resistance, constants of capacitance of the arterial and venous system and the factor limiting the flow into the heart on the other follow from the description of the model. The conclusion is drawn that peripheral resistance is to be considered mainly as a regulator of blood flow rather than as a primary determinant of arterial blood pressure.

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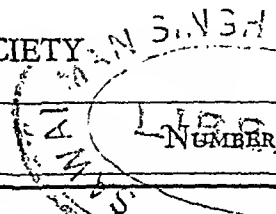
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LITHIUM: ITS EFFECT ON HUMAN SPERMATOZOA AND TESTICULAR TISSUE AND UPON RATS IN VIVO

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DURING an investigation of phosphatase activity in human spermatozoa (1), we discovered, quite accidentally, that the lithium ion, in relatively low concentration, has an inhibiting effect on the metabolism and motility of human spermatozoa (2). The results to be reported here indicate that lithium is worthy of more intensive study, particularly in view of its recent prominence in therapeutics and the knowledge that, in certain circumstances, it may be highly toxic (3).

Lithium has received relatively little attention from physiologists and pharmacologists as evidenced by the sparsity of references in the respective fields. The embryological literature, however, as reviewed by Needham (4) contains many studies which show that lithium has profound effects on the early embryo, particularly in producing exogastrulation and failure of development of the notochord. The nature of its action remains highly obscure. Indeed, Needham (4) in summarizing his review of the effects of lithium states "that up to the present time (1944) a biochemical effect of lithium equivalent in magnitude to its morphological effect has been sought for in vain."

In the pharmacological field, an experimental study of lithium toxicity in cats (5) showed that the chloride (60 mg/kg.) caused extreme muscular weakness, severe gastro-intestinal symptoms and death in from 24 hours to 3 days. In the human being (6) a total of 8 gm. of lithium chloride taken over a period of 28 hours produced marked muscular and general prostration, vertigo and eye and ear symptoms over a period of several days but no gastro-intestinal symptoms. Its absorption and excretion in the human subject has been studied (7) and while no toxic effects were reported, it was shown that soluble Li^+ salts added to a diet are readily absorbed and quantitatively excreted in the urine. It is not absorbed well from a natural diet.

More recently, a commercial preparation³ containing 25 per cent LiCl has been used widely as

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³ This product has since been withdrawn from the market.

a salt substitute in low sodium diets. A report (3) in publication indicates that, in certain circumstances, LiCl produces hyperirritability and pronounced muscular weakness. One death has been attributed to its use, the patient in question exhibiting the above symptoms prior to coma and death.

There are few references to the effect of lithium on tissue metabolism. The studies on embryos (4) were concentrated on respiratory metabolism with no definite results reported. The growth of yeast and yeast fermentation (8) are not appreciably affected by the ion though other ions in the same series (K, Cs, Rb) exert a considerable stimulating effect on yeast growth. Recently, Ponder (9) has shown that human red cells are most fragile in LiCl. In the field of tissue metabolism, there are references (10) to the effect of various cations on glycolysis and respiration but so far as can be determined, no studies on Li^+ have been done.

The following experiments are confined to the effect of Li^+ on the metabolic and motile behavior of human spermatozoa, its effect on the metabolism of rat testicular tissue and the acute and chronic effects of subcutaneous injection of LiCl in rats.

METHODS

The aerobic and anaerobic lactic acid production and respiration of the tissues were measured by the Warburg technique over a period of several hours, at 38°C . by methods already described in detail (11). It should be emphasized that the spermatozoa, in all experiments, were removed from the seminal fluid by centrifugation and re-suspended in Ringer-glucose-bicarbonate for the glycolysis determinations and in Ringer-glucose-phosphate for measurement of oxygen consumption. The testicular tissue was obtained from rats killed by a blow on the head. Tissue aliquots of approximately 150 mg. were weighed on a torsion balance and transferred to Warburg manometer vessels containing measured amounts of the various Ringer glucose solutions. The lithium (in the form of LiCl) was added to the spermatozoa suspension and the testicular tissue from the side-arms of the manometer vessels after equilibration at 38°C . with the appropriate gas mixtures and just prior to the first (zero time) manometric reading. Manometric lactic acid production was checked chemically, in certain experiments, by the Barker and Summerson technique (12).

The *in vivo* experiments were conducted on mature male rats (average weight 250 gm.), maintained on the normal laboratory diet. The LiCl was injected subcutaneously and the rats left under constant observation subsequently.

EXPERIMENTS

The spermatozoa were obtained from normal human semen by centrifugation and re-suspended in the appropriate Ringer-glucose solutions for measurements of oxygen consumption, aerobic and anaerobic glycolysis. The standard cell suspension volume used was 1 cc. per Warburg vessel and the total spermatozoon count ranged from 100 to 150 million cells/cc. The motile activity of the cells and the percentage of cells active was determined prior to equilibration of the vessels with the appropriate gas mixtures. The LiCl was placed in the side-arm of the vessels and added to the main vessel containing the cell suspension at the conclusion of the gas equilibration and about 5 minutes previous to the zero manometric reading. Thereafter, readings were taken at 30-minute intervals and the usual duration of each experiment was 3 to 4 hours. Two control suspensions were run in every

experiment and motile examinations were made in these and in the experimental suspensions at the end of each run. Figure 1 shows the effects upon the anaerobic and aerobic lactic production and motile activity of the cells in the presence of the designated molarities of LiCl.

The curves are representative of both aerobic and anaerobic glycolysis since, as shown previously (13), there is virtually no quantitative difference in the two glycolytic mechanisms. The molarities indicated are final molarities in the system.

It will be seen that the inhibition of glycolysis appears rapidly in each dilution used but that in any one concentration, the glycolysis thereafter proceeds steadily at a new, lower steady state rather than undergoing increasing inhibition.

In the highest Li^+ concentration indicated (0.025M) the total reduction in lactic acid production over the 4-hour period is 40 per cent and this inhibition is remarkably constant in any given experiment using the same Li^+ concentration. However, two- or three-fold increases in the Li^+ concentration above this level do not produce progressively greater inhibitions. The highest inhibition attained was 60 per cent at a concentration of 0.10M. However, complete failure of the motile activity of the spermatozoa (fig. 1) is attained at relatively low concentrations of the ion. As would be expected, there is good correlation between the inhibition of glycolysis and the failure of motility, though it will be evident that a considerable glycolytic activity remains when virtually all motile activity has ceased.

Effect of LiCl on oxygen consumption of spermatozoa. In contrast to the effect of lithium on glycolysis, similar concentrations of the ion have no effect on the oxygen consumption. In a series of experiments using concentrations of Li^+ which would depress glycolysis about 50 per cent, no striking effect on O_2 consumption could be observed.

Effect on metabolism of rat testicular tissue. Experiments were designed 1) to determine if similar effects of the ion could be produced in testicular tissue, and 2) if lithium affected the production of normal spermatozoa in the mature animal. For obvious reasons, it is not possible readily to obtain normal human testicular tissue. Furthermore, it was not considered advisable to risk human *in vivo* experiments. The rat was selected as being the most appropriate experimental animal, since breeding experiments can be performed within a short space of time and the metabolic activity of the testicular tissue of that animal is known. One of the characteristics of rat testicular tissue is a considerable production of lactic acid under aerobic conditions.

The effect of lithium on the rat testicular tissue is summarized in table 1. The concentrations of the ion inhibiting the glycolysis and motility of human spermatozoa have no detectable effect on the respiration and anaerobic glycolysis of testicular tissue but do have definite stimulating effect on aerobic glycolysis. The experiments recorded in the table were measured manometrically, but chemical checks on the lactic acid production were made in certain experiments to be certain that the extra acid produced was lactic acid. Though perfect chemical checks were not obtained, it is certain that at least 85 per cent of the extra aerobic acid produced in the presence of lithium is lactic acid. These results are in distinct contrast to the inhibiting effect of the ion on the glycolysis of spermatozoa.

Up to isotonic concentrations of lithium only aerobic glycolysis is affected, the highest elevation of the latter in this range being about 75 per cent. On the hypertonic side (0.2M), the ion begins to produce a slight depression of oxygen consumption, though relatively high concentrations inhibit respiration to a considerable degree. Anaerobic glycolysis is not affected appreciably in any concentration tested. These findings are of more than ordinary interest and will be discussed later.

Effect of lithium on rats in vivo. As a further extension of the investigations of Li^+ on the germinal tissues, LiCl was injected subcutaneously into mature male rats to determine 1) the acute effects of the ion and 2) if spermatogenesis was af-

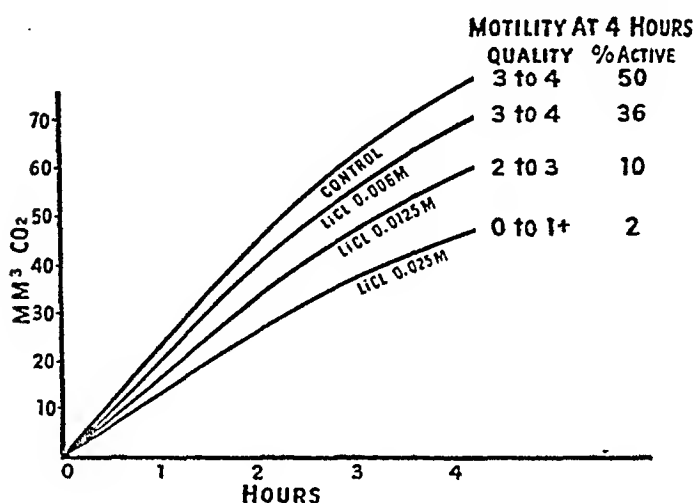


Fig. 1. EFFECT OF LITHIUM on motility and lactic acid production of human spermatozoa.

TABLE 1. EFFECT OF LiCl ON METABOLISM OF RAT TESTICULAR TISSUE

MOLARITY OF LiCl	EFFECT ON RESPIRATION	EFFECT ON ANAEROBIC GLYCOLYSIS	EFFECT ON AEROBIC GLYCOLYSIS, %
0.01	0	0	+10
0.02	0	0	+30
0.05	0	0	+75
0.20	-10%	0	+100-125
0.40	-30%	0	+140
1.00	?	+10%	+200

ected. The injection dose was selected to correspond on a weight basis with the experiments of Good (5) on cats, namely 60 mg/kg. Eight animals were injected, 2 of which were injected with Li^+ as LiCl , 2 as LiCO_3 , 2 as LiSO_4 and 2 controls with NaCl . No acute symptoms were noted at this injection level and the dose was doubled (120 mg/kg.). The results are shown in table 2. Within 24 hours, rat 1 developed marked hyper-irritability and weakness of the hind legs. It died shortly thereafter. Rat 2 developed exactly the same symptoms within 24 hours but did not die. Several hours later, the animal showed generalized weakness with loss of hyper-irritability and spontaneous movement. It recovered uneventfully. However, when this animal was given a dose corresponding to 180 mg/kg., it became markedly hyperactive after 22 hours, and developed spontaneous generalized tre-

mors. Pronounced weakness of the hind legs developed in 26 hours and respiration ceased at 27 hours. *Rats* 3 and 4 were discarded because the LiCO_3 was insoluble and produced skin ulcerations at the site of injection.

Rats 5 and 6 showed exactly the same symptoms as *rats* 1 and 2 and died at 28 hours. As might be expected the rats injected with NaCl showed no signs of abnormal behavior. Gross inspection of the organs of the experimental animals at autopsy showed no obvious injury, except that bright, red blood mixed with the feces was found in the bowel of *rat* 1. Similarly, microscopic examination of the organs of the experimental animals showed no obvious lesions. Admittedly, this is a small number of experimental animals, but since all 4 succumbed with the same symptoms and since we were more interested in the chronic effects of lithium on the

TABLE 2. EFFECT OF LITHIUM ON MALE RATS

RAT	ORIGINAL WEIGHT	AUTOPSY WEIGHT	DOSE OF Li/ DAY	TOTAL DOSE BEFORE AUTOPSY	TOXIC SIGNS
	gm.	gm.	mg.	mg.	
1	275	265	1		Anesthetic death
2	280	300	1	34	None
3	270	280	1	34	None
4	250	270	3	102	None
5	280	300	3	102	None
6	265	270	3	102	None
7	215	230	5	170	32nd day present ¹
8	220	270	5	170	31st day present ¹
9	220	220	5	170	None
11	310	270	10	240	14th day present ¹
12	290	250	10	240	18th day present ¹
13	320	310	15	120	7th day present ¹
14	240		15	135	Died 11th day present ¹
15	350	340	15	105	Died 8th day

¹ The signs of toxicity were invariably increased reflex excitability followed by paralysis of the hind limbs and death. One animal was seen to die with labored respirations of diminishing rate. Several of the toxic animals developed profuse nasal discharges and harsh rasping respirations.

reproductive system, the acute study was concluded at this point and chronic experiments begun.

Table 2 shows the effect of daily injection of subacute doses of LiCl in male rats. The daily administration ranged from 1 to 15 mg. subcutaneously for periods ranging up to 34 days. Approximately 14 days after the first injection, the males were placed with mature females of known breeding capacity and the mating results noted.

Up to the 3-mg. daily dose level and a total injection level of 102 mg., no toxic symptoms were noted in the animals and each male conceived normal litters with 2 females. Furthermore, each animal gained weight during the experiment. At the 5-mg. daily dose level and a total amount injected of 170 mg., toxic symptoms appeared in 2 of the 3 animals tested on the 31st and 32nd days, respectively. The animal showing toxic symptoms on the 32nd day conceived 2 normal litters, while the other animal exhibiting toxicity failed to impregnate in 2 cases. The 3rd ani-

mal of this group did not show toxic symptoms but, on the other hand, failed to conceive litters in 2 females.

At the 10- and 15-mg. daily dose level, every animal developed typical toxic symptoms ranging from the 8th to the 18th day, and all lost weight. Two of the 3 animals on the 15-mg. dose level died on the 8th and 11th days respectively. The 8-day animal did not show any symptoms prior to death. The remaining animal of this group started to show symptoms on the 7th day, when a total of 105 mg. had been injected. Of the 5 animals in the 10- to 15-mg. dose level group, only 1 sired a normal litter, and this animal failed to do so with another female. But 1 animal (no. 14) died before exposure to the female and another (no. 15) died on the day of exposure to the female. The remaining 2 animals of this group (11 and 13) probably were too weak for copulation and their failure to reproduce can thus be accounted for. In confirmation of this, the testes of all the rats were sectioned and examined for observations of the germinal epithelium. In all cases, the latter appeared normal with an abundance of mature spermatozoa in the tubules. As in the acute experiments, microscopic examination of the other organs disclosed no obvious lesions.

DISCUSSION

From these experiments, it is obvious that the lithium ion has effects *in vitro* and *in vivo* which can be construed as highly toxic. At the present time, it is difficult to explain the widely different effects of the ion on the glycolysis of the spermatozoa and upon that of rat testicular tissue. First, it should be emphasized that lithium inhibits both the aerobic and anaerobic pathways of the degradation of glucose to lactic acid in the spermatozoa, whereas it affects only the aerobic glycolysis in testicular tissue and that in stimulatory fashion instead of inhibitory. It should be remembered, however, that a suspension of spermatozoa consists of homologous cells, whereas testicular tissue is composed of several cell types, the germinal epithelium and interstitial tissue being examples of widely different forms so far as morphology and function are concerned. Furthermore, we have already demonstrated (13) that it is difficult to dissociate the aerobic and anaerobic glycolysis of human spermatozoa. There is no evidence that the two pathways can be affected selectively and no Pasteur effect is apparent in these cells. But there are certain analogies between the carbohydrate metabolism of human spermatozoa and of muscle (13) which would indicate that the enzymatic components are probably similar if not the same. The effect of certain cations, particularly K^+ , has been demonstrated in brain (10). The aerobic glycolysis of that tissue is raised above the anaerobic glycolysis in the presence of excess K^+ , whereas the anaerobic glycolysis is inhibited. These workers did not use lithium and it is not likely that the effects of lithium we have found in testicular tissue and in the spermatozoa are analogous. In fact, we can state that the K^+ concentration in the environment of the spermatozoa can be increased at least one hundred fold without affecting the glycolysis or motility to any appreciable extent (14). Human spermatozoa are able to withstand relatively large changes in their osmotic environment, both on the hypotonic and hypertonic sides (14). It is therefore more suggestive of a specific ionic effect that

the lithium ion, in such low concentrations, can produce the effects described here on the spermatozoa.

In regard to the acute and chronic effects of lithium on normal rats, muscular weakness is one of the striking symptoms. This is true also in human toxicity (3). In fact, the symptoms produced in the rat are strikingly similar to those found in the human subject whose sodium chloride intake has been replaced by lithium chloride. It is significant, perhaps, that if one extrapolates, on a weight-for-weight basis, the chronic lithium dosage producing the symptoms in rats to the human being, the theoretical intake necessary to produce the human symptoms would be from 1.3 to 2.3 gm. daily. This corresponds rather closely with the intake in the human cases reported. Good (5) has shown in the cat that by far the greatest amount of Li^+ retained in that animal was to be found in the muscles. Our evidence in the rat points definitely to retention of Li, and the most pronounced symptoms produced are muscular in nature. If the analogy from the spermatozoa be projected, it would not be difficult to assume an effect on carbohydrate metabolism in muscle when a critical level of lithium is reached. This would be critical if cardiac muscle were affected in similar fashion, since lithium as a salt substitute is likely to be used as a salt substitute in many cases of cardiac insufficiency. It is distinctly possible, on the other hand, that lithium produces effects on the central nervous system which would produce certain of the effects found both in the rat and human subject.

SUMMARY

Lithium chloride, in low concentration, inhibits the aerobic and anaerobic glycolysis of human spermatozoa and destroys their motile activity. The lithium ion, in contrast, stimulates the aerobic lactic acid production of rat testicular tissue without affecting the anaerobic glycolysis or the respiration of that tissue. The acute and chronic effects of lithium chloride injected subcutaneously into male rats are described. It is shown that the symptoms induced in rats are strikingly similar to those found in lithium toxicity in the human being.

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ACETALDEHYDE METABOLISM AND LIVER CHANGES IN DOGS MAINTAINED ON A PURIFIED DIET¹

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THERE are present in animal tissues at least four different enzyme systems capable of utilizing acetaldehyde as a substrate. These are: 1) one or more flavo-proteins similar to xanthine-aldehyde oxidase; 2) an aldehyde mutase requiring DPN as a hydrogen carrier; 3) animal carboxylase containing diphosphothiamine; and 4) aldolase. All of these could theoretically be involved in the metabolism of acetaldehyde, but previous studies (1) have indicated that the carboxylase system does not play a major role in this process. The mutase and oxidase are concentrated in the liver, and the liver is known to be a major site of acetaldehyde metabolism (1). Since both of these enzyme systems are dependent upon dietary factors, it seemed possible that a study of acetaldehyde metabolism during niacin or riboflavin deficiency would aid in assessing the relative importance of each of these enzymes in the metabolism of acetaldehyde.

The experiments herein reported resulted from such an attempted investigation, but it soon became evident that the abnormal acetaldehyde metabolism which resulted from the feeding of a purified diet was unrelated to niacin or riboflavin deficiency. When dogs were fed a purified diet containing adequate niacin and riboflavin, the rate of disappearance of administered acetaldehyde was slowed. A degenerative change in the liver, characterized by a loss of cytoplasmic staining properties and by a swelling of the liver cords with obliteration of the sinuses, was found in those dogs showing an abnormal acetaldehyde disappearance curve. Other liver function tests were normal.

EXPERIMENTAL

The basic diet for this study is shown in table 1. In some of the experiments the tocopherols were omitted without apparent effect. The nicotinic acid-deficient diet was prepared by omitting the nicotinic acid. The low protein diet contained 8 per cent casein and 39 per cent sucrose. The high protein diet contained 35 per cent casein and 24 per cent glucose. In one series of experiments an attempt was made to inhibit intestinal bacterial synthesis of growth factors by injecting the B vitamins subcutaneously rather than incorporating them in the diet. In this latter series the foodstuffs and choline were fed orally while the subcutaneous vitamin mix-

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ture² administered weekly provided 0.5 mg. each of thiamine, riboflavine and pyridoxine, 2.5 mg. of calcium pantothenate, and 12.5 mg. nicotinamide per kg. body weight per week.

Before young adult dogs were placed on one of these diets and at arbitrary intervals during the dietary regime, the acetaldehyde disappearance curve was determined. This 'load test' was run as follows. A 4 per cent solution of acetaldehyde was prepared by diluting 51.3 cc of freshly distilled acetaldehyde to one liter with cold 0.8 per cent NaCl. The dog was lightly anesthetized with 0.8 cc/kg. of 3.25 per cent nembutal intravenously because of the irritation accompanying the acetaldehyde administration. Previous studies have shown no effect of anesthesia on the rate of acetaldehyde metabolism. Two cc/kg.³ of the 4 per cent acetaldehyde were injected intravenously over exactly 3 minutes, and blood samples were removed from the jugular vein at exactly 3, 5, 8 and sometimes 12 minutes after completing the injection. A 1:10 tungstic acid filtrate was rapidly prepared from each oxalated blood sample, and the protein precipitate was removed by centrifuging in a stoppered tube in the cold room. Loss of acetaldehyde from the samples by volatilization was minimized by keeping all tubes stoppered and in an ice bath at all times possible.

TABLE 1. COMPOSITION OF SYNTHETIC BASAL DIET FOR ACETALDEHYDE STUDIES

Casein ¹	19 gm.%	Nicotinic acid.....	2.5
Cod liver oil.....	2	Calcium pantothenate.....	0.5
Cottonseed oil (Wesson).....	7	Riboflavin.....	0.4
Salt mix (Phillips and Hart).....	4	Thiamine chloride.....	0.2
Sucrose.....	28	Pyridoxine hydrochloride.....	0.2
Glucose.....	40		
Choline chloride.....	100 mg.%		
34% mixed tocopherols ²	10		

¹ Vitamin Test, General Biochemicals, Inc. ² Distillation Products, Inc.

The acetaldehyde in the filtrate was distilled and determined by the method of Stotz (2).

Liver total lipid was determined by weighing the chloroform-soluble extractives from dried liver. Bromsulfalein excretion (3), prothrombin time (4), and serum alkaline phosphatase (5) were determined as indicated. Biopsy or fresh autopsy sections of the liver were fixed in 10 per cent formalin for histological study.

RESULTS

Figure 1 shows some typical acetaldehyde disappearance curves. The two lower lines represent the range in 'normal' dogs (up to 6 mg. % at the 3-minute point), while the zone between curves II and III is considered a moderate elevation; blood levels above III are clearly abnormal, while those in the region of V and VI are often associated with a fatal termination. For convenience in subsequent discussions the blood acetaldehyde level at the 3-minute point will be used to indicate the location of the acetaldehyde disappearance curve on figure 1.

² Solu-B with added pyridoxine, Upjohn Company.

³ Early doses of 3 cc/kg. of 4% acetaldehyde produced a high percentage of fatalities among the deficient dogs.

Most dogs tested at weekly, bi-weekly, or longer intervals continued to show normal acetaldehyde disappearance curves until the first appearance of a delayed metabolism, after which high curves were consistently obtained. Moderately elevated blood acetaldehyde levels were not considered significant because of occasional spontaneous fluctuations between the normal and moderately elevated zones. Very rarely a dog would show a single high curve, and then spontaneously drop back into the normal range. Considerable spontaneous variation was noted from week to week in the exact level of the abnormal curves; no particular significance could be attached to this type of fluctuation within the abnormal zone.

Of 46 mongrel adult dogs chosen at random and studied in this investigation, the initial acetaldehyde disappearance curve was normal in 35, moderately elevated (7-11 mg.%) in 8, and definitely high (16-25 mg.%) in 3 dogs. Twenty-seven of the normal dogs were maintained on one of the purified diets for at least 8 weeks; 15 of these eventually developed high curves (16-25 mg.%), 3 were moderately elevated (9-11 mg.%), and the remaining 9 dogs continued to show normal acetal-

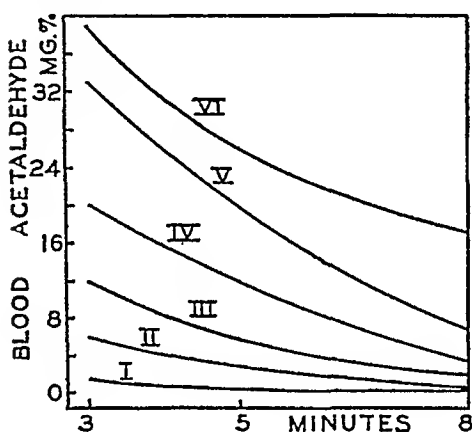


Fig. 1. TYPICAL ACETALDEHYDE DISAPPEARANCE CURVES IN DOGS. Two cc/kg. of 4% acetaldehyde were injected i.v. over 3 minutes; blood samples were analyzed for acetaldehyde 3, 5, and 8 minutes after completing the injection. Disappearance curves falling in the zone between curves I and II are normal; above curve III is clearly abnormal.

dehyde disappearance curves throughout the dietary regime. Four out of 5 dogs starting with moderately elevated levels developed high curves (14-31 mg.%) when maintained on a purified diet. One of the dogs starting with a high curve was given a variety of dietary factors over a period of 6 months without permanently lowering it, and this dog eventually died following a very high blood acetaldehyde (38 mg.%).

Ten deaths in this series were attributed to the acetaldehyde administration, and all of the deaths followed high acetaldehyde curves (usually 30-40 mg.%). Three deaths resulted from respiratory failure while the experiments were being run, but this cause of death was eliminated in the others by routinely giving artificial respiration to all dogs showing signs of a high curve (slow heart rate and respiratory failure). The latter deaths occurred after the blood acetaldehyde had fallen to reasonably low or negligible levels. Some of the dogs died following the first or second high acetaldehyde curve, while others survived repeated high blood acetaldehyde levels.

The basal diet, the nicotinic acid-deficient diet and the subcutaneous vitamin regime all gave comparable results in producing an abnormal acetaldehyde metabolism. Five dogs maintained on the basal diet developed high curves within approx-

imately 12 weeks; 6 other dogs were maintained on the same diet for approximately 20 weeks without developing this abnormality. Maintenance on a nicotinic acid-deficient diet (supplying single nicotinic acid injections as the deficiency became severe in order to maintain life) resulted in high curves in 4 dogs within 4 to 9 weeks, while 4 other dogs continued to show a normal acetaldehyde disappearance after 9 to 17 weeks. Of 5 dogs that were given both the basal and nicotinic acid-deficient rations at different times during their dietary history, 2 developed high curves in 17 to 19 weeks; 2 remained normal for at least 26 weeks, and 1 dog was continued for 100 weeks without showing abnormal acetaldehyde metabolism. Feeding the basal diet to which 1 per cent sulfasuxidine had been added produced a high curve in one dog in 11 weeks, but not in another dog that was continued for 19 weeks. Of 6 dogs that were fed the purified foodstuffs orally and given the vitamin supplement subcutaneously, 4 developed high curves within 10 to 22 weeks, while the other 2 remained relatively normal for 26 weeks.

Five of the dogs maintained on the basal or subcutaneous vitamin diet for 20 to 26 weeks without developing an abnormal acetaldehyde metabolism were then placed on the low protein diet. Two of the dogs developed high curves in 3 and 11 weeks respectively; the other three still had normal disappearance rates after 11 to 17 weeks on low protein. A sixth dog that did not develop a high acetaldehyde curve during 100 weeks on the basal diet did so after three weeks on the low protein diet. These results indicate that a low protein diet aids in establishing the abnormal acetaldehyde metabolism.

Practically all of the dogs on the basal or subcutaneous vitamin diet increased in weight. Changes in body weight on the low protein diet were small. Body weights fluctuated widely on the nicotinic acid-deficient diet as nicotinic acid was withheld or administered. The appearance of abnormal acetaldehyde curves could not be correlated with such weight changes. Both normal and abnormal disappearance rates were observed at all phases of the growth response in different dogs.

Liver Studies. A number of dogs showing normal acetaldehyde disappearance curves (2–5 mg. %) were tested for bromsulfalein excretion, plasma prothrombin time and serum alkaline phosphatase just before they were put on the basal ration or the subcutaneous vitamin regime. After subsisting on the purified diet for approximately 14 weeks, the acetaldehyde test was repeated, and from the group were chosen 5 dogs showing definitely high acetaldehyde curves (20–25 mg. %) and 3 dogs still showing normal curves (4–6 mg. %). The above liver-function tests were repeated in these dogs, and a liver biopsy was then removed from each under aseptic conditions.

Bromsulfalein retention in the group of dogs at the start of the experiment averaged 1.55 mg. %, and did not change significantly during the dietary period. The prothrombin time of 12½ per cent plasma was essentially the same (22 sec.) in both the normal and high acetaldehyde group. Serum alkaline phosphatase averaged 1.15 units in the group before starting the experiment; the normal group did not change during the dietary period, but each of the dogs that developed a high curve also showed a perceptible increase in serum phosphatase, the abnormal group averaging 2.48 units at the time of biopsy. Liver lipid and water content were normal (5.7%

lipid, 31% total solids) in a dog running a high acetaldehyde curve (28 mg. %), and there was no gross or histological evidence of fatty livers in any of the adult dogs maintained on these diets.

Histological Studies. The livers were studied histologically without reference to biochemical changes. After fixation in 10 per cent formalin, paraffin sections were made and stained simultaneously with hematoxylin and eosin. The sections were grouped into three categories: normal, definitely abnormal and intermediate. The principal features studied were cell size, liver cord arrangement, sinusoid size and cytoplasmic staining qualities. All of these were altered in the abnormal and intermediate groups, the difference being one of degree.

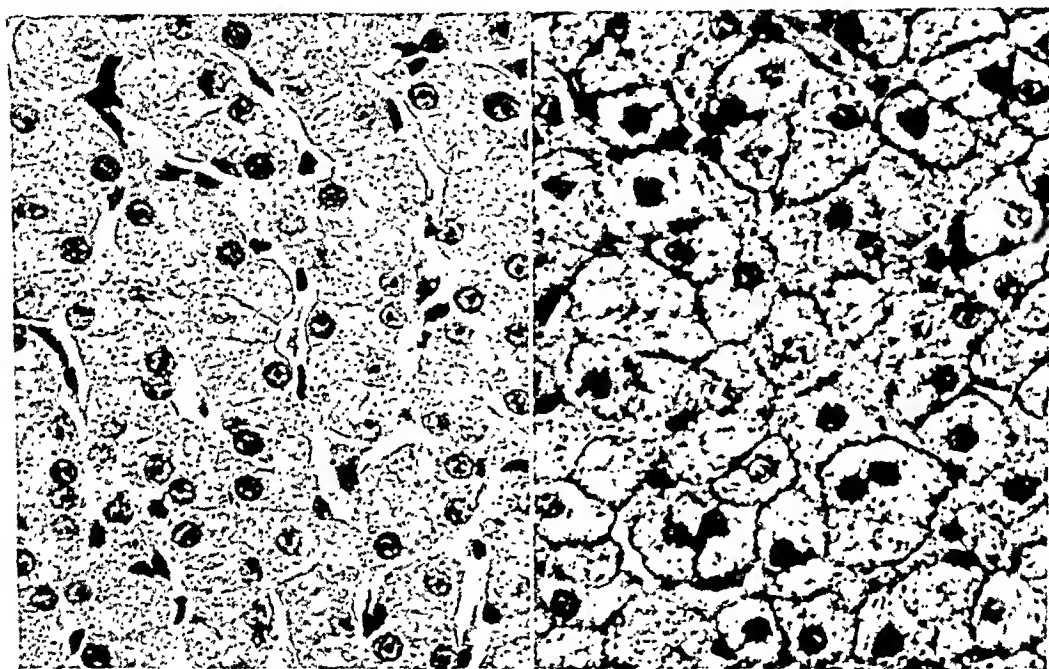


Fig. 2 (left). NORMAL DOG LIVER. $\times 525$.

Fig. 3 (right). LIVER FROM DOG maintained on synthetic basal diet. $\times 525$. Section shows severe hydropic degeneration.

The normal dog liver (figure 2) has fairly straight liver cords radiating from the central vein, regular sinusoids and uniform cells with distinct boundaries and fine eosinophilic granules in the cytoplasm. The livers which were classified as abnormal (figure 3) appeared disorganized. Liver cords were indistinguishable and sinusoids were not apparent. The outline of the individual cells was very distinct because of a characteristically deeply stained material which appeared to be the cell membrane. Because they were considerably larger than normal they encroached upon the sinusoids and either distorted or obliterated them. The cytoplasm was rarified except for a few scattered threads and granules of eosin stained material. There were no distinct vacuoles. Stains for fat (Herxheimer Scarlet Red) and glycogen (Best's Carmine) did not show any appreciable abnormalities.

Many of the dogs maintained on the basal ration for several months showed some degree of this so-called hydropic degeneration of the liver, but the livers of all

dogs were not invariably altered pathologically. Occasional dogs have been maintained on the diet for long periods of time without developing abnormal changes in the liver. There was a general tendency for abnormal acetaldehyde disappearance curves to be associated with these degenerative changes in the liver. Of 6 dogs that continued to show normal acetaldehyde metabolism while being maintained on the diet for 3 months or longer, 3 had normal livers and 3 showed moderate degeneration. Of 7 dogs that gave elevated acetaldehyde curves at the time of liver biopsy, 4 showed moderate degenerative changes in the liver and 3 had severe hydropic degeneration. The appearance of high acetaldehyde curves and liver pathology bore no relationship to the amount of acetaldehyde previously administered.

Effect of Dietary Supplements. Dietary supplements were evaluated in terms of the effect obtained on the elevated acetaldehyde disappearance curve. Following the first appearance of a high acetaldehyde curve, additional tests were made at weekly intervals to establish the consistent nature of the abnormal change. The dietary supplement was then given orally or subcutaneously for periods varying from one to 12 weeks, and the acetaldehyde test was repeated at weekly or bi-weekly intervals during the period of supplementation. When the supplement was ineffective in restoring the curve to normal, it was replaced by another test substance. A positive response to the supplement was considered to be significant when the initial high curves, exceeding 12 mg. per cent for several weeks prior to testing, were reduced to normal (6 mg. % or less) and maintained there during the period of supplementation for at least 3 to 4 weeks. According to this criterion, a high casein diet cured 3 of 5 dogs tested. Inositol gave some positive, but erratic effects without permanent cures. Other substances tested were without effect.

Table 2 summarizes the results obtained in some of the tests with dietary supplements, particularly protein and inositol. Methionine was also tested and found negative in 2 dogs that were later found to be refractory to the high protein diet. The high protein diet had to be fed for 5 to 6 weeks to some dogs in order to obtain an effect. The results are clear in assigning a beneficial role to large amounts of casein in this condition.

The beneficial response to inositol was erratic, unpredictable and temporary. Reasonably normal curves first produced by inositol administration were not maintained in spite of continued inositol administration. In some dogs inositol had no beneficial effect at all; in others it was beneficial the first time it was administered but subsequent high curves in the same dog were unaffected by repeated administration of inositol either orally or subcutaneously.

Negative tests were obtained with biotin, p-aminobenzoic, folic acid, tocopherols, menadione, ascorbic acid, cystine, ergostanyl acetate, a 4-fold increase in choline, and a mixture of trace elements. The results with protein and inositol demonstrate that the condition is sometimes reversible by dietary means, but some dogs have been refractory to natural supplements such as Wilson's liver L, fresh liver, Brewer's yeast, milk and horse meat. For example, 2 dogs maintained their body weight on a diet of raw milk exclusively for 6 weeks, but continued to show high acetaldehyde curves throughout this period. This failure to respond to natural supplements may represent an irreversible stage of liver damage. Some of the dogs

tests are equivocal because there is no assurance that any dietary factor would have yielded a cure at the time of the test.

Alcohol and Acetoin. Two dogs averaging 20.2 kg. were maintained on the nicotinic acid-deficient diet for 8 weeks. At this point they averaged 16.0 kg. and both showed high acetaldehyde curves. The average rate of alcohol metabolism was determined (6) to be 10.6 mg. % per hour following an oral dose of 15 cc. of 19 per cent (by volume) alcohol per kg. After continuing on the same diet with a large excess of nicotinic acid for two weeks, body weights increased to 18.2 kg., and the

TABLE 2. EFFECT OF DIETARY SUPPLEMENTS IN RESTORING THE ACETALDEHYDE DISAPPEARANCE CURVE TO NORMAL

WEEKS OF DIET AND TYPE ¹	AcH CURVES BEFORE ADMIN. OF TEST SUBSTANCE ²	SUBSTANCE TESTED		AcH CURVES DURING ADMIN. OF TEST SUBSTANCE ²
		Name	Mg/Kg. Body Wt. Daily	
9 N	* 16—28	Inositol	22	8—6
		PABA	7	
		Biotin	0.16	
100 B + 5L	23—29—31	High prot.	Ad. lib.	**24**4—5—5
4 N	* *—27	Biotin	0.17	41 (fatal)
15 S	17—11—20	Inositol	56	6—6—10—13
20 B + 5L	13—17—15	High prot.	Ad. lib.	**8—5—5—6—4
17 B	* 22—13	Inositol	81	*7—21—10**22
		High prot.	Ad. lib.	*31**6—7—5—4
16 B	* 25—21	Inositol	80	**28
		Methionine	160	27—8*18*30
		High prot.	Ad. lib.	**31**11*10
15 B	* 25—17	Inositol	66	*9—12—27**18
24 V	24—14—15	Tocopherols	47	11—15—18
		Vit. K	1.2	
24 V	18—16—15	P. A. factor	$\frac{1}{2}$ unit	11—10—10
14 V	* 20—26	Inositol	83	*35—35
		Methionine	166	37—37*31*26
		High prot.	Ad. lib.	**35**29*14
13 B	* 20—20	Inositol	63	*37—9—21**13

¹ Diet abbreviations: B = basal; N = nicotinic acid deficient; L = low protein; S = sulfasuxidine; V = vitamins subcutaneously.

² Each figure refers to mg. % blood acetaldehyde at 3-minute point, determined at weekly intervals. * indicates a week in which no curve was run.

alcohol disappearance rate increased to 15.5 mg. per cent per hour; the acetaldehyde disappearance curves remained high. The change in the rate of alcohol metabolism following treatment with nicotinic acid was probably significant, but it should be noted that the rate observed during the deficiency was not exceptionally low as compared with the average expected of a group of 'normal' dogs. Blood acetoin following the administration of 3 cc. of 4 per cent acetaldehyde per kg. was determined (7) in several nicotinic acid-deficient dogs showing delayed acetaldehyde disappearance curves. As expected from earlier results (1), the high blood acetaldehyde (35 mg. %) was accompanied by high levels of blood acetoin (0.5–0.7 mg. %).

Other Species. Acetaldehyde metabolism was studied in rats by injecting intra-peritoneally 0.5 cc. of 4 per cent acetaldehyde per 100-gm. body weight. Blood samples obtained by decapitation were analyzed, and the averages of the individual results obtained were 18, 9 and 2.5 mg. % acetaldehyde respectively at the 3-, 5-, and 10-minute points. This same normal curve was obtained in rats fed Purina dog chow for 41 and 75 days after weaning, and in a parallel group of rats fed the purified nicotinic acid deficient ration. Other weanling male rats were placed on the purified basal diet previously described and killed at monthly intervals. Histological sections of the livers stained with eosin and hemotoxylin were normal throughout the 4-month dietary regime; liver lipids were similarly normal, averaging 15.6 per cent of the total solids.

Two monkeys that were being killed after several years of vicarious existence in the laboratory were subjected to the same acetaldehyde load test used in the dogs. In comparison with the curves obtained in dogs (fig. 1), one monkey had a slightly elevated curve (8 mg. %) while the other was markedly elevated (24 mg. %).

DISCUSSION

Some, but not all, adult dogs maintained on a typical purified diet develop a defect in acetaldehyde metabolism that is reflected in a delayed disappearance curve. The nature of the dietary deficiency responsible for this effect has not been established clearly. A low casein diet aids in establishing the pathology, and a high casein diet restores a certain percentage of animals to normal in this respect. Nevertheless, it is difficult to believe that this syndrome is a manifestation of protein deficiency per se. It can be produced in adult dogs on a 19 per cent casein diet (supplying approximately 18% of the calories) and none of the dogs show protein deficiency changes in body weight or plasma proteins. The response to the high casein diet is slow. The casein could be a carrier for some unknown nutritional factor, or the protein content of the diet could play an accessory rôle in the production of some other deficiency.

The altered histology of the liver is called hydropic degeneration despite the small or negligible changes in water content. Such degenerative changes in the liver were previously described in dogs fed a low protein diet (8, 9). The present study indicates that protein deficiency is not the only factor capable of producing hydropic degeneration, since it was obtained with diets containing 19 per cent casein. The altered acetaldehyde metabolism could have been due to changes in the gross architecture of the liver or to a specific biochemical lesion in one of the enzyme systems concerned with acetaldehyde metabolism.

SUMMARY

Fifty to 60 per cent of the adult dogs maintained on a purified diet containing the usual B-complex vitamins developed a defect in acetaldehyde metabolism that could be demonstrated by an acetaldehyde load test. A low casein diet helped to produce this deficiency but it could be obtained on a 19 per cent casein intake. The delayed acetaldehyde disappearance curve was not associated with body-weight changes but was correlated to some extent with the appearance of hydropic degenera-

tion in the liver. Other evidence of liver damage was not prominent; liver fat, brom-sulfalein excretion and prothrombin times were normal; serum alkaline phosphatase may have been slightly elevated. Once this defect was produced, the acetaldehyde disappearance curve could be restored to normal in some dogs by the prolonged administration of a 35 per cent casein diet; it was also benefited sporadically by inositol. Disappearance curves in other dogs could not be restored to normal even by feeding natural foodstuffs for moderate periods of time.

Weanling rats grown on the same ration did not show any abnormalities in acetaldehyde metabolism or liver histology.

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EFFECT OF CALORIC RESTRICTION ON THE ADRENAL RESPONSE OF OVARIECTOMIZED C₃H MICE

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WOOLLEY, Fekete and Little (1) reported that certain strains of mice develop nodular hyperplasia of the adrenal cortex following ovariectomy. Kirschbaum, Frantz and Williams (2) found that such changes occur spontaneously in the NH strain in relation to the early ovarian failure seen in this strain. The change can be induced even earlier by ovariectomy. These facts suggest that the adrenal may be responding to pituitary stimulation caused by low estrogen levels in the body fluid. As the adrenal changes develop the uterus and vagina show increasing evidence of estrogen stimulation. The adrenal is, presumably, the source of the estrogen.

These facts have a bearing on caloric restriction since a number of investigators have interpreted their findings as indicating that dietary restriction inhibits the pituitary causing various degrees of gonadal failure; see Moore and Samuels (3); Werner (4); Mulinos and Pomerantz (5); Huseby, Ball and Visscher (6); and others cited in the above papers.

In this study an attempt was made to determine whether the partial or total anterior pituitary inhibition which is believed to prevail in caloric restriction is of such a nature as to prevent the adrenal changes known to follow ovariectomy in certain strains of mice. It was also important to determine whether restriction influenced estrogen production in case the adrenal changes developed in mice on restriction.

Since the effects of pure caloric restriction on the C₃H mouse have been extensively investigated in this laboratory (6, 7) and since this strain shows the typical adrenal response following ovariectomy it was used exclusively in this study.

MATERIALS AND METHODS

C₃H mice were ovariectomized at 21 to 23 days of age. They were housed individually in an air-conditioned room. The diet used was that described by Visscher *et al.* in their study on the incidence of mammary carcinoma. The controls had unlimited access to food. The restricted mice were fed a pellet formed in a brass mold calibrated to measure the required amount for one day. One group was restricted to approximately 66 per cent of the calories eaten by the controls; another group was restricted to approximately 50 per cent. Tap water was continually available to both restricted and control groups. Each group contained at least 50 mice.

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It should be noted that the restricted mice received approximately the same amount of protein, vitamins and salts as the controls. Animals were weighed each week. Vaginal smears were made by the lavage method. Sections were made of the uterus, vagina and adrenals.

General observations. Restricted mice were uniformly more active than full-fed mice. They were also more irritable when handled. The restricted ration was promptly eaten by a mouse in good health. There were some perianal infections in the full-fed groups which yielded to prompt washing with boric acid solution and painting with tincture of metaphen. There was no evidence of poor health in restricted animals as compared to controls. Fur, skin, eyes, nose and mouth showed no abnormality.

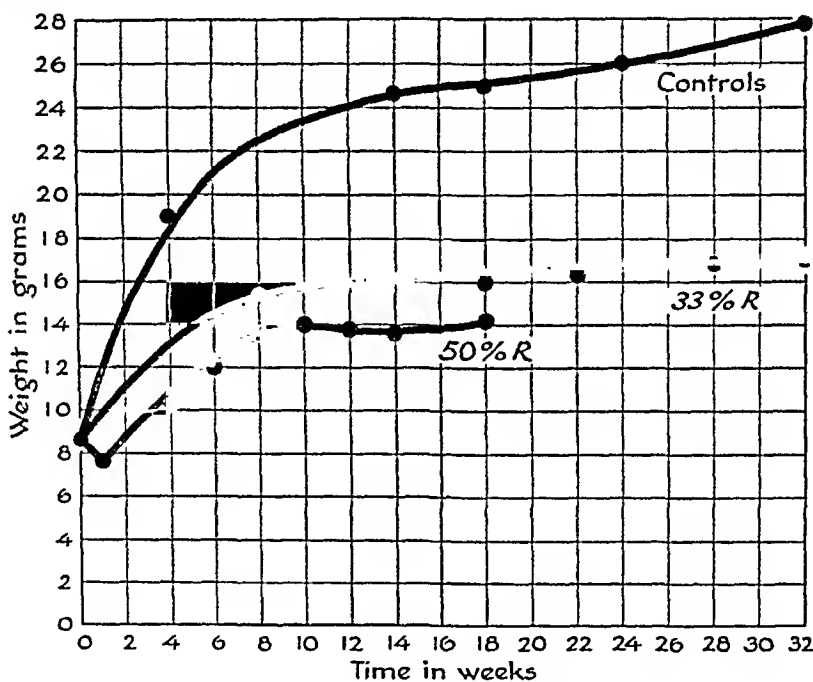


Fig. 1. GRAPH OF BODY WEIGHT of mice fed *ad libitum*, at 33% and 50% caloric restriction.

Growth rate. In figure 1 the rate of growth of restricted and control group is shown up to the 32nd week. The rapid growth phase persists longer in the controls. In the group restricted to 50 per cent there is a loss in the first week which is recovered in the second. Thereafter the curve for this group resembles that of the group under less severe restriction but is at a lower level.

Vaginal smears. The restricted animals never developed a positive smear. At 37 days after ovariectomy the controls began to show evidence of stimulation; in 4 months they showed a dense, mixed smear composed of leucocytes, epithelial and cornified cells. Beyond this time there is no essential change in the character of the smear. The cellular content of the smear is non-cyclic in contrast to normal estrus cycles seen in the intact, full-fed animal.

Adrenals. By 37 days after ovariectomy histological changes could be observed in both restricted and control groups. The changes were further advanced in the controls. There was proliferation of small, dark subcapsular cells which invade the

cortex. When this type of cell surrounds large, clear cells the result is that described by Woolley and Little (8) and noted by Kirschbaum, Frantz and Williams (2) in the NH strain. At the 3-month stage the changes were further developed in both restricted and controls. At this time there was no qualitative difference between the two groups although there was a larger fraction of the cortex involved in the controls. The X zone disappears very early in that part of the gland showing the above histological changes. Grossly the glands showed white or yellowish areas, sometimes bulging on the surface.

Uterus. By the time the vaginal smears showed the dense, mixed picture the uterus of the control mouse was grossly enlarged and hyperemic while that of the restricted animal was completely atrophic. Sections of the control organ showed evidences of estrogenic stimulation, i.e., tall columnar epithelium, infolding of the luminal surface, numerous well developed glands, loose stroma, etc. In the restricted mouse the histologic picture is one of hypoplasia.

Vagina. In the restricted mouse the vagina remained very small with usually not more than two rows of epithelial cells, except deep in the folds. The full-fed animal showed a well-developed vagina at 99 days with an epithelium many layers deep. Mucification was prominent in some animals with some cornification. Later stages showed more pronounced cornification.

Refeeding. Five of the ovariectomized restricted mice were fed *ad libitum* after 12 weeks of restriction at which time their weight varied from 17.7 gm. to 20.0 gm. They came into subestrus after 29 to 35 days of full feeding at body weights between 22.2 gm. and 26.4 gm.

DISCUSSION

This experiment was based on the assumption that the controls would eat on the average 2.66 gm. per day after the second week. They actually ate slightly more than the expected amount. The restricted animals therefore were subjected to a caloric restriction very slightly in excess of that planned.

The outstanding difference between the full-fed and restricted ovariectomized mouse is that the former develops evidence of estrogenic stimulation while in the latter the uterus and vagina remain atrophic. Both develop qualitatively identical histological changes in the adrenals. Since our unpublished experiments show that the restricted mouse is as sensitive to estrogens, in vaginal and uterine response, as the full-fed animal one is forced to believe that it does not produce enough estrogen to cause stimulation.

The concept of pseudohypophysectomy advanced by earlier workers seems adequate to explain the failure of ovarian function seen in the restricted, intact mouse since the response to gonadotropin or pituitary implants is prompt. Stated in its simplest form it means that restriction inhibits at least that function of the pituitary which controls the ovary.

No such simple explanation is adequate, however, when applied to the facts observed in the tumor-bearing, restricted castrate. This animal has adrenal cortical tumors qualitatively identical with those seen in the full-fed mouse but there are no signs of estrogen production. It is true that controls usually show more tumor

tissue in the cortex than do restricted mice. However, we have seen control animals with the usual evidences of estrogenic stimulation but having less tumor tissue than seen in some restricted mice failing to show such evidences. Hence it is improbable that the lesser amount of tumor tissue seen in the restricted animal explains failure to produce estrogens.

In the present state of knowledge caution must be used in interpreting these facts. It seems very probable that the tumors are the site of estrogen production in full-fed castrates of certain strains and in the NH mouse where they occur spontaneously. However, the fact that the tumors occur in the restricted castrate without evidence of hormone production makes it clear that the factors which are responsible for the histological change and for the secretory activity are not identical. Whether suppressed pituitary activity in the case of the restricted mice is the determining difference cannot be answered finally on the basis of these experiments. Our efforts to bring about estrus in the restricted tumor-bearing mouse with gonadotropin (9) and with adrenocorticotropin (10) were not successful. Likewise we were not able to produce estrus with fresh pituitary implants (unpublished data). The results of Boutwell, Brush and Rusch (11) indicate that restriction may inhibit one pituitary function while enhancing another.

SUMMARY

The ovariectomized C₃H mouse develops adrenal cortical adenomas whether full-fed or restricted. The restricted tumor-bearing mouse fails to show stimulation of the uterus and vagina while the full-fed animal shows constant stimulation following the development of histological changes in the adrenal cortex. Where the caloric block to estrogen production takes place and what rôle the pituitary plays remain unsolved problems.

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SECRETION OF INSULIN AND OF A HYPERGLYCEMIC SUBSTANCE STUDIED BY MEANS OF PANCREATIC-FEMORAL CROSS-CIRCULATION EXPERIMENTS¹

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THE hypoglycemic phase of the normal glucose tolerance curve is generally attributed to excessive compensatory reduction of the blood sugar level, brought into play by the hyperglycemic phase. Investigators, however, do not agree as to whether this is accomplished by stimulation of insulin production (1-5) or through a protracted inhibition of liver glycogenolysis (6). The solution of this problem is important for academic and practical reasons, for if one believes that hyperglycemia stimulates the insular tissue and that a continuous over-activity may result in exhaustion, one must control both diabetes mellitus (7-8) and hyperinsulinism (9) with 'painstaking care' (7). On the other hand, if one believes that changes in the rate of liver glycogenolysis is the paramount factor, the strict control of the blood sugar level and of glycosuria may become less important.

METHODS

The problem was investigated by means of pancreatic-femoral anastomoses. Seventy dogs were used. All animals were fasted for 16 hours preceding the experiment and were anesthetized with sodium amytal (70 mg/kg. intramuscularly, plus a single 50 to 100-mg. dose intravenously, if needed). This barbiturate was selected because it is reported to have little or no effect on blood sugar concentration (10, 11). The experiments were performed as follows. A donor *dog A* was prepared by cannulating the pancreaticoduodenal and one of the femoral veins. The other femoral vein was exposed for withdrawal of blood samples. A recipient *dog B*, smaller than *dog A*, was prepared by cannulating the femoral vein and artery on one side and exposing the femoral vein on the other. The blood pressure of the dogs was measured with mercury manometers connected with the carotid arteries. Having thus prepared the animals, a control sample of blood was taken, the animals were heparinized,² the pancreaticoduodenal vein of *dog A* was connected with the femoral vein of *dog B* and the femoral artery of *dog B* with the femoral vein of *dog A*. The anastomoses were made with transparent polyethylene tubing and the free flow of blood ascertained by injecting a minute bubble of air into the tubing before every blood sampling. The pancreatic-femoral anastomosis was kept open continuously. The return flow from the artery of *B* to the vein of *A* was opened as needed to maintain arterial blood pressures of both dogs approximately equal to their initial values. Four samples of blood were taken at 15-minute intervals and, after this control period, 5 cc. of a 20-per cent solution (1 gm.) of glucose per kilo body weight or the same volume of iso-osmotic (4%) saline were rapidly injected into the exposed femoral vein of *dog A*. Blood samples were taken at 15-minute intervals for 75 minutes following the injection, the anastomoses were then disconnected and the sampling continued 15 minutes later and then every 30 minutes for 2 more hours. Blood sugar was determined in duplicate according to the method of Folin and Malmrose (12). In some control experiments a branch of the mesenteric vein of *dog A*, about equal in size to its

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² Heparin was generously supplied by the Wilson Laboratories of Chicago.

pancreaticoduodenal vein, was used instead of the latter. To minimize any difference in the rate of blood flow, cannulas of similar size were used in all experiments.

In other experiments alloxan diabetic dogs were used. The animals had received 70 mg. of alloxan per kilo of body weight by intravenous injection at least 3 days before the experiment and had had glycosuria for at least 2 days.

The statistical significance of all pertinent data was computed and expressed as 'Probability' (P).

RESULTS

The results are presented in the form of average blood sugar curves. Figure 1 represents the glucose tolerance curve of intact anesthetized and heparinized dogs.

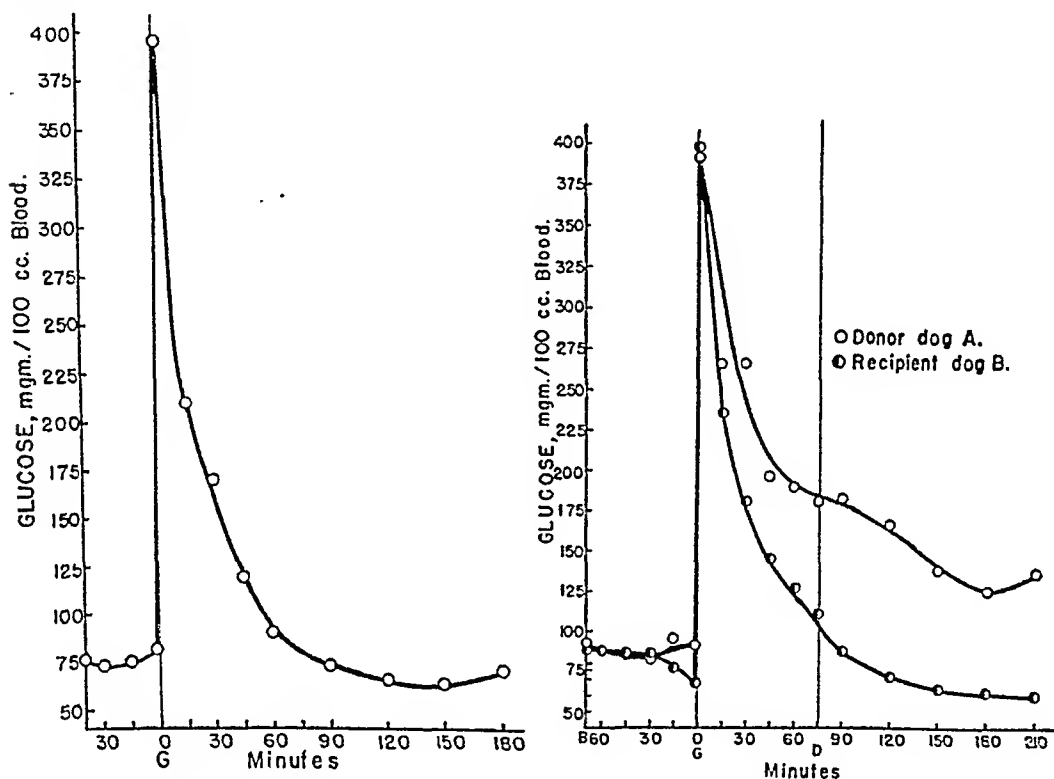


Fig. 1 (left). GLUCOSE TOLERANCE CURVE. Normal dogs. Average of 4 experiments. G: intravenous injection of 20% glucose solution (1 gm/kg. body weight).

Fig. 2 (right). PANCREATIC-FEMORAL ANASTOMOSIS. Normal dogs. Average of 3 experiments. B: before opening the anastomosis; G: intravenous injection of 20% glucose (1 gm/kg. body weight) into both dogs; D: anastomosis disconnected.

It demonstrates that, under the experimental conditions used, the carbohydrate metabolism of the animals was not altered significantly, as a normal curve could be obtained.

Figure 2 shows that when dogs A and B are connected by means of the pancreatic-femoral anastomosis and glucose is injected into both dogs simultaneously, the tolerance curve of dog A resembles a diabetic curve, whereas that of dog B is normal. The difference in the behavior of the two dogs shows that at least part of the insulin produced by the pancreas of dog A can be transferred to dog B and that the preparation used is suitable for this type of study.

Figure 3 shows the results of experiments in which dogs A and B were connected by a pancreatic-femoral anastomosis and glucose was injected only into the donor

dog A. The following phenomena will be observed: *a)* During the control period preceding the injection, the blood sugar of both dogs remained practically constant; *b)* following the injection of glucose into *A* the blood sugar of *B* started to decline sharply within 30 minutes, reaching a minimum value in from 60 to 90 minutes. This sharp decline was observed in all experiments and was even more marked than it would appear in the average curve. The latter is somewhat flattened because the beginning of the decline and the lowest blood sugar concentration did not happen at exactly the same time in all experiments.³ The blood sugar of *B* remained low for about 2 hours after the two dogs had been disconnected, then gradually returned toward normal levels.

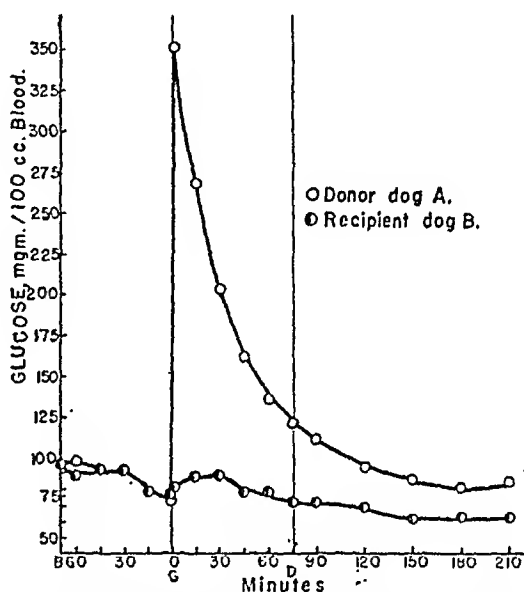
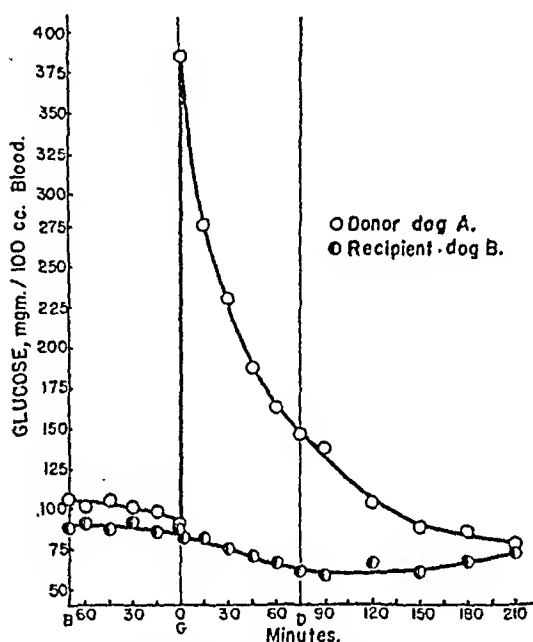


Fig. 3 (*left*). PANCREATIC-FEMORAL ANASTOMOSIS. Normal dogs. Average of 5 experiments. G: intravenous injection of 20% glucose (1 gm/kg. body weight) into the donor dog.

Fig. 4 (*right*). MESENTERIC-FEMORAL ANASTOMOSIS. Normal dogs. Average of 6 experiments.

It will be further noted that the decline in the blood sugar concentration of *dog B* followed the rise in blood sugar concentration of *dog A*, at a time when the glucose present in the pancreatic blood of *A* flowing into *B* tended to raise the glycemia of the latter rather than lower it. The hypoglycemia observed in *dog B* thus acquired even greater meaning. The fact that the decline in the blood sugar of *B* did not start until about 15 to 30 minutes after the blood glucose of *A* had been raised by the injection, that it reached minimum values in from 30 to 60 minutes and that it lasted for more than 2 hours after the animals had been disconnected suggests that the phenomenon is due to overproduction of insulin by the pancreas of *dog A* following the injection of glucose. The following experiments were performed to confirm this tentative interpretation.

It was necessary to rule out the possibility that the described phenomena may have been due to non-specific actions of the injection per se on the pancreas of the

³ The difference between the mean glucose concentration before and after the injection of glucose is statistically highly significant ($P < .01$).

donor dog, such as: *a*) some hemodynamic disturbance produced by the volume of fluid injected or *b*) the hypertonicity of the solution. For this purpose experiments were performed in which the donor dog received hypertonic (4%) saline instead of glucose.

The sudden hypoglycemia of *dog B* was not observed. Although a gradual decline in the blood sugar of both dogs was noted in the average curve, it was absent in 3 of the 5 donor dogs and in 2 of the 5 recipients.⁴

To rule out the possibility that the hypoglycemia of *dog B* might have been due to the blood of *A* per se, rather than to its insulin content, six experiments were performed in which the dogs were connected with a mesenteric-femoral instead of a pancreatic-femoral anastomosis.

Figure 4 shows that following the injection of glucose into *dog A* the blood sugar of *dog B* increased instead of decreasing.⁵ This phenomenon is probably due to the high glucose content of the blood flowing from *dog A* to *dog B* and adds significance to the hypoglycemia observed in the dogs receiving pancreatic blood from their donors. It will be noted that, following the rise, the blood sugar of *dog B* tended to decline below the original level. This may have been due to the preceding hyperglycemia or to the tendency to hypoglycemia observed after the injection of saline for which we have no satisfactory explanation. The latter decline is, however, readily distinguishable from the sudden hypoglycemia shown in figure 1.

The possible non-specific effect of blood transfusion was further ruled out by the results of 3 experiments in which an alloxan diabetic dog was used as donor of pancreatic blood. One can see (fig. 5) that, following the injection of glucose into *dog A*, the blood sugar of *dog B* rose instead of declining, probably because of the glucose content of the pancreatic blood of *dog A* which contained no insulin.⁶ The tolerance curve of *A* is strongly diabetic.

It appears, therefore, that the hypoglycemia noted in *dog B* receiving pancreatic blood from a normal donor cannot be explained by a non-specific effect of the injection or of the blood transfusion and was probably due to stimulation of insulin production by *dog A* following the injection of glucose.

If one compares figure 4 with figure 5 one will observe that the rise in blood sugar of a *dog B* receiving blood from the pancreatic vein of an alloxan diabetic donor (fig. 5) was much greater and lasted much longer than that of a *dog B* receiving blood from the mesenteric vein of a normal donor (fig. 4).⁷ The difference in

⁴ Statistical analysis showed that the decline in blood sugar concentration of *dog B* after saline injection is not significant ($P = .6$). It also showed that the difference between the mean decrease in blood sugar of *dog B* after injecting glucose into *dog A* (fig. 3) and the mean decrease in blood sugar of *dog B* after injecting saline into *dog A* is significant. ($.02 > P > .05$). The difference between the mean of the blood sugar concentrations reached in the 2 experiments is highly significant ($P > .01$).

⁵ The increase in blood sugar concentration is statistically significant ($P = .05$).

⁶ The difference in mean blood sugar concentration of *dog B* before and after the injection of glucose into *dog A* is highly significant ($P > .01$). The difference between the mean rise in blood sugar noted in this experiment is significantly different from the mean decrease shown in fig. 3 ($.02 > P > .05$).

⁷ The difference between the mean increases in blood sugar concentration is highly significant ($P < .01$).

response was too great to be explained by the higher blood sugar concentration of the alloxan diabetic donor as compared to the normal donor and suggests the possibility that the pancreas of the former may have secreted a hyperglycemic principle not masked by the simultaneous secretion of insulin. This possibility is made stronger by the fact that when *dog B* received blood from the mesenteric instead of the pancreaticoduodenal vein of an alloxan diabetic donor, its blood sugar behaved very much like that of a dog receiving blood from the mesenteric vein of a normal dog (fig. 6).⁸

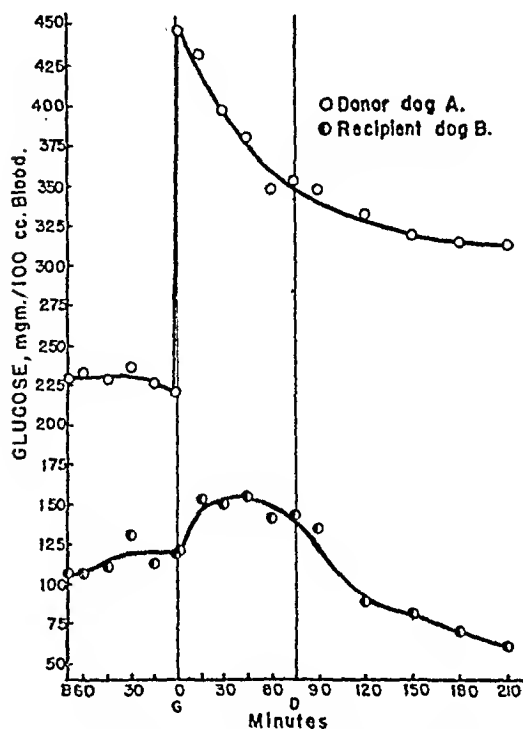


Fig. 5 (left). PANCREATIC-FEMORAL ANASTOMOSIS. Alloxan diabetic donor. Normal recipient. Average of 3 experiments.

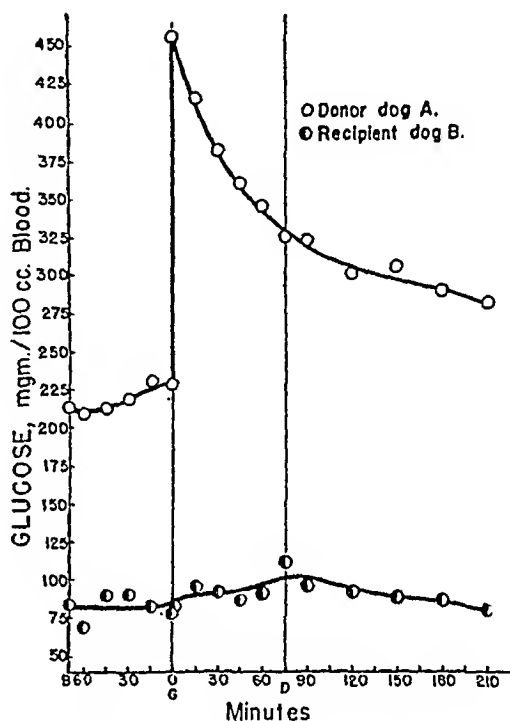


Fig. 6 (right). MESENTERIC-FEMORAL ANASTOMOSIS. Alloxan diabetic donor. Normal recipient. Average of 2 experiments.

DISCUSSION

The following observations support the view that hyperglycemia stimulates insulin production: *a*) Carbohydrate tolerance is decreased after fasting or low carbohydrate diets and is restored after a carbohydrate meal (13, 14). *b*) Carbohydrate tolerance is decreased after prolonged insulin administration which probably inhibits insulin secretion (15-17). *c*) Diabetes can be produced in normal animals by feeding a high carbohydrate diet (18) or by keeping them hyperglycemic with large amounts of glucose (19). *d*) Animals can be made permanently diabetic by glucose after partial pancreatectomy (Sandmeyer's Diabetes) (20) or after partial injury to

⁸ The rise in blood sugar concentration of *dog B* is not significant ($P = .3$) whereas the difference between the mean rise observed in fig. 5 and that observed in fig. 6 is highly significant ($P < .01$).

the pancreas with anterior pituitary extract (21) or alloxan (22). Apparently every time more carbohydrate is available to the animal, the insulin requirement and secretion increase. This may overtax and finally exhaust the islet cells. *e*) When the pancreas is protected by exogenous insulin (21, 23), or by preventing hyperglycemia with phlorizin (24), *beta* cell degeneration and diabetes can be prevented. *f*) A hypoglycemic crisis follows a carbohydrate meal in patients with hyperinsulinism (25).

Further evidence of the effects of hyperglycemia in stimulating insulin secretion was obtained by comparative measurements of blood sugar (26) or of insulin (27) concentration in the femoral and pancreaticoduodenal arteries after the injection of glucose. It was also found that hyperglycemic dog blood produces hypoglycemia in the mouse (28) (although this may be a non-specific effect of heterologous blood) and that glucose injected into the pancreatic artery reduces the systemic blood sugar (29-31). Zunz and La Barre (32) anastomosed the pancreatic vein of a *dog A* with the jugular of a *dog B* and found that the injection of glucose into *A* produced hypoglycemia in *B*. Their conclusions are based on only 3 experiments and there were no adequate blood sugar determinations preceding the injection of glucose. The effect of blood other than pancreatic was not studied. Furthermore, the hypoglycemia obtained was rather mild perhaps because the flow of blood through the vascular anastomosis was minimal, due to lack of anticoagulant and to the constriction produced by the Payr cannula. Recently Anderson and Long (33) found that the amount of insulin secreted by a perfused pancreas increases when the perfusing fluid contains glucose. Results which would not support the hypothesis that glucose stimulates insulin secretion have also been reported. Gellhorn, Feldman and Allen (34) were unable to alter the concentration of insulin in the blood by injecting glucose. Geiger and Houssay and Lewis and Foglia (quoted in 31) found that the results of Grafe and Meythaler (26, see above) were not specific as they could be obtained by using saline or urea instead of glucose. Furthermore, according to Soskin (6), the theory that insulin secretion is stimulated by hyperglycemia is not tenable in view of the experiments by himself and collaborators on the depancreatized and the hepatectomized dog. According to Soskin the hyperglycemia would inhibit the release of glucose from the liver until the blood reaches hypoglycemic levels. Not all authors concur with this point of view (Best in 6, 35, 36). Furthermore, recent evidence (37) would indicate that in man the inhibition of the release of hepatic glucose induced by hyperglycemia ceases when the blood sugar concentration is still at the relatively high value of 170 mg/100 cc.

The results of Zunz and La Barre and of other investigators quoted above were confirmed and extended by us, using the cross-circulation technique. This technique obviates the objection of using an animal of a different species for the determination of the insulin content of the blood, subjects the pancreas to a minimal trauma and maintains the recipient or insulin-detector dog nearly intact.

The evidence indicates that the hypoglycemia observed in the recipient dog following the injection of glucose into the donor is probably the result of a specific increase of insulin secretion in the donor dog brought about by the hyperglycemia. The order of magnitude of the hypoglycemia in *dog B* is similar to that observed in the normal glucose tolerance curve. It appears likely, therefore, that in the intact

normal animal the concentration of glucose in the blood regulates insulin secretion and by so doing it regulates itself. Hyperglycemia would stimulate the pancreas, the excess insulin would increase glucose utilization in the peripheral tissues (38-40) and decrease liver glucogenolysis (6). Although other mechanisms may be available to secure the regulation of the blood sugar in the depancreatized animal (41), in the intact animal the pancreas probably plays a primary rôle.

The hyperglycemia observed in the dog receiving pancreatic blood from an alloxan diabetic donor may be due to a pancreatic hyperglycemic substance similar to that found in most insulin preparations (42-44).

While this manuscript was being prepared, there appeared a paper by Sutherland and De Duve (45) showing that the *beta* cells of the islet tissue can be destroyed by alloxan without loss of the hyperglycemic factor. The hyperglycemia obtained by these authors injecting alloxan-pancreas extracts is comparable in time and duration to the hyperglycemia observed in our recipient dogs receiving pancreatic blood from an alloxanized donor. It appears, therefore, that this pancreatic principle derives from the alloxan-resistant portion of the pancreas. Sutherland and De Duve also report the interesting observation that blood destroys the hyperglycemic factor *in vitro*. Our cross-circulation experiments appear to indicate that this destruction does not occur *in vivo*, at least during the duration of the experiment. Its independence of the *beta* cells may explain why the depancreatized dog requires less insulin than the dog in which diabetes has been produced not by removal of the organ, but by injury to the *beta* cells with alloxan or anterior pituitary extract (46). It may also help explain why a totally depancreatized man requires less insulin than a moderately severe diabetic (47). Experiments designed to demonstrate the presence of a hyperglycemic principle in the pancreas of alloxan diabetic dogs are in progress.

SUMMARY

In 35 cross-circulation experiments, anastomoses were made between the pancreaticoduodenal or the mesenteric vein of a donor *dog A* and the femoral vein of a recipient *dog B*. A return circulation was obtained by establishing a limited flow of blood between the femoral artery of *dog B* and the femoral vein of *dog A*.

The blood sugar of a dog receiving pancreatic blood from a normal donor injected with glucose decreases sharply reaching a minimum in 30 to 60 minutes and gradually returning toward normal about 2 hours after the anastomosis has been disconnected. This sharp decrease is similar to the hypoglycemic phase of the normal glucose tolerance curve. Both phenomena are consistent with the hypothesis that a rise in blood sugar stimulates the secretion of insulin and by so doing regulates itself.

When saline is injected instead of glucose the glycemia of *dog B* does not decrease significantly. When the transfusing blood derives from the mesenteric vein of a normal donor the blood sugar of the recipient dog increases instead of decreasing. The increase in the blood sugar concentration of the recipient dog is even more marked and sustained if the anastomosis is made with the pancreatic but not with the mesenteric vein of an alloxan diabetic donor. The latter hyperglycemia sug-

gests that the pancreatic blood of the alloxan diabetic dog contains a hyperglycemic substance produced by the alloxan resistant portion of the pancreas and active *in vivo*.

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COMPARATIVE EFFECT OF INTRAVENOUS GLUCOSE AND ADRENALIN ON BLOOD FLOW, OXYGEN UTILIZATION AND GLUCOSE RETENTION BY HIND LEG TISSUES OF ANESTHETIZED CATS

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IN A recent report (1) it was shown that although the carbohydrate plethora of an adrenalin hyperglycemia is invariably accompanied by increased transfer of sugar from blood to peripheral (hind leg) tissues, this increase in tissue sugar uptake or retention was usually accompanied by a decrease rather than an increase in tissue oxygen consumption.

Insofar as a specific calorigenic action of adrenalin itself might be involved, this result was not unexpected since we have been repeatedly unable to detect a locus for it in peripheral tissue of intact animals (2-4) and there is considerable evidence, too extensive to be documented here, that it is abrogated by hepatectomy or evisceration.

Insofar, however, as the carbohydrate plethora itself might be involved, the result was unexpected since the locus of its specific dynamic action is presumably in the peripheral tissues themselves; it not only occurs after hepatectomy or evisceration (5, 6) but apparently is even intensified thereby (7).

If the only effect on oxygen consumption in the experiments referred to had been a depression, it might have been concluded that adrenalin directly suppressed the specific dynamic action of the very carbohydrate plethora evoked by it. The suppression of oxygen consumption of the leg tissues was, however, not invariable; there were enough instances in which it increased to suggest that whichever effect occurred must depend on some indirect effect of the intravenously injected adrenalin and it was suggested that this might be its effect on blood flow within the limb. Whenever this was decreased (which apparently could only result from such intense local vasoconstriction as to offset the effect of a presumably elevated arterial blood pressure) oxygen consumption was decreased also. When blood flow through the leg was increased it was assumed this might occur in two ways: 1) sufficient elevation of blood pressure to drive an increased amount of blood through still patent channels and in spite of extensive local vasoconstriction; and 2) actual local vasodilatation if the effective adrenalin concentration reaching the leg was of a minimally effective magnitude. In (1) the oxygen supply to and its utilization by the leg tissues would be as effectively curtailed as in those experiments in which the blood flow was actually diminished; in (2) oxygen supply would be and its utilization presumably could be increased. This, at least, seemed to provide a possible explanation for the finding that: a) oxygen consumption always decreased if blood flow decreased; b) never increased unless blood flow increased; and c) might at times be decreased even though blood flow was increased.

This interpretation is based on assumptions which seem consonant with generally accepted premises as to the vascular readjustments effected by adrenalin. It was obviously desirable, however, to have direct measurement under the conditions of these experiments of the actual change of blood pressure and of the degree of vaso-

constriction or dilatation (plethysmograph) coincident with the measured oxygen consumption by and flow rate through the leg tissues. In addition, it was desired to compare the effects of adrenalin on these variables with those accompanying a hyperglycemia of approximately equal magnitude produced by glucose injection alone. For although there are scattered observations in the literature as to tissue glucose uptake resulting from a glucose hyperglycemia, there are none known to us which permit a quantitative comparison of this with the effect of an adrenalin hyperglycemia of equal degree. Nor do we know of any measurement of the effect of glucose injections of this magnitude on arterial blood pressure, or blood flow and oxygen utilization by peripheral (leg) tissues.

It is the purpose of this report, then, to compare the effect on arterial blood pressure and leg volume, and the change in blood flow, sugar retention and oxygen consumption of the leg which result from: *a*) intravenously injected adrenalin; and *b*) glucose injected intravenously in such amounts as to effect a comparable hyperglycemia.

PROCEDURE

All of the work was done on cats anesthetized with dial-urethane (Ciba). Intravenous injection of adrenalin or glucose was by way of the cannulated left jugular vein. Arterial blood pressure was recorded from the right carotid by means of a Hürthle membrane manometer. Heparin was administered intravenously to prevent clotting.

Arterial blood samples for determination of arterial glucose, oxygen and hemoglobin concentrations were obtained from a cannula in the right iliac artery.

Venous blood from the left hind leg was obtained from a special cannula in the right iliac vein into which flowed all the blood from the left hind leg when the vena cava was clamped just above its origin. As the blood entered this cannula it was kept at a fixed mark on a vertical side arm by withdrawal into a tightly fitting syringe the rate of filling of which was timed with a stopwatch, thus measuring the rate of blood flow from the left leg. Most of this blood would be immediately reinjected and only enough retained in the syringe for determination of venous glucose, oxygen and hemoglobin concentrations. These values and those of a simultaneously obtained arterial blood sample provided the arterio-venous differences in concentrations which, together with the measured rate of blood flow, permitted calculation of the sugar retained and oxygen used by the tissues of the left hind leg per unit time.

After these preparations were complete the left hind leg was enclosed in a plethysmograph fitting as high up toward the hip as possible. An air-tight seal with the shaved skin was uniformly easily obtained with Unna paste. The plethysmograph was insulated from sudden variations of room temperature by thick wrapping with cotton batting. Variation of leg volume was transmitted by air to a Marey tambour and recorded on the smoked kymograph paper synchronously with the arterial blood pressure.

Adrenalin was employed in only one dosage, 0.004 mg/kg. of body weight per minute in one cubic centimeter; to obtain this, proper dilution of Parke-Davis 1:1000 adrenalin chloride solution was made with isotonic NaCl solution immediately before use. Injection was by a hand-operated syringe into the jugular vein and timed with

a stopwatch at a rate as nearly as possible of 1 cc/min. for 5 minutes; it was begun immediately after securing a pair of simultaneous normal arterial and venous blood samples; and its immediate effect was determined by another pair of blood samples taken during the terminal portion of the 5th minute of injection. After-effects were determined by samples obtained 5, 15 and 30 minutes after the end of the 5-minute injection period.

Glucose hyperglycemia was desired to be as nearly as possible equal to that induced by the adrenalin; for the rate of adrenalin injection used here this had been found in previous work (1) to be an average increase of about 85 mg. per cent. Preliminary trial indicated that an increased blood sugar level of approximately this amount would be caused by intravenous injection of 0.25 gm. of glucose per kg. of body weight in 5 minutes (0.05 gm/kg/min. for 5 min.). For reasons not significant here, this amount was dissolved in 2.5 cc. of distilled water and injected, as was the adrenalin, into the jugular vein by a hand-operated syringe at the rate of 0.5 cc/min. for 5 minutes. Blood samples were taken as described for adrenalin.

Blood sugar was determined by the method of Hagedorn and Jensen; hemoglobin determinations for assessment of change of blood hydration were by the colorimetric method of Cohen and Smith; blood oxygen content was obtained by the manometric method of Van Slyke and Neill; careful precaution was taken to prevent alteration of gas content of the blood samples during collection and while awaiting analysis.

RESULTS

The data to be presented were derived from 25 experiments with adrenalin and an equal number with glucose injected at the rates and in the quantities described above.

Blood hydration. As in our previous work, and as have others, we find that venous blood from the leg is consistently (and in this work invariably) more concentrated than the arterial blood going to it as measured by the change in hemoglobin concentration and expressed as oxygen capacity. Also, as before, the degree of concentration varies greatly from one experiment to another so it is not surprising to find that the average for the 50 normals of this series is somewhat different (higher) than reported previously by us; i.e., the average oxygen capacity of venous blood in this series was 0.81 vol. per cent greater than arterial. But, again, in conformity with previous findings, intravenously administered adrenalin affects this very little and in the present instance not at all, the difference being exactly the same at the end of the 5-minute injection period. Nor did glucose infusion change this blood-tissue water exchange to any marked degree, merely diminishing it slightly so that the oxygen capacity of venous blood was only 0.74 volume per cent greater than arterial at the end of the 5-minute intravenous injection. When, later on, the effect of the glucose infusion on total leg volume is considered this evidence of an only slightly diminished loss of water from arterial blood to the tissues will have to be reconsidered.

The oxygen contents of venous blood samples have in all cases been corrected for the concurrently indicated change in blood hydration in this as in our previous work. For there is apparently no reason to doubt that, since the bulk of the oxygen

is in the corpuscles, this dehydration of the blood as it passes through the leg makes the apparent oxygen content of venous too high in comparison with that of arterial blood.

Blood sugar, however, now appears to us to be in a different category. Whereas, in our previous work venous blood values were similarly corrected for the change in hydration, it now seems that until there is definite evidence to the contrary, it is safer to assume that the water leaving the blood will carry small molecules with it without any change in concentration. Whether applied or not this correction makes no difference qualitatively and in most instances little quantitatively.

Hyperglycemia. Intravenous injection of adrenalin at the rate and for the time employed here (0.004 mg/kg/min. for 5 min.) produced an increase in arterial blood sugar level or a hyperglycemia of 77 mg. per cent as the average of the 25 experiments. This checks well with our previous work in which the average of 14 injections at this same rate was 85 mg. per cent.

Since it was desired to raise the blood sugar level as nearly as possible an exactly equal amount by intravenous glucose infusion, the preliminary selection of 0.05 gm/kg/min. for 5 minutes as the rate of administration proved to be singularly appropriate; the average elevation of arterial blood sugar level thus induced at the end of the 5-minute infusion period for the 25 experiments was 82 mg. per cent—a remarkably close approximation to the effect produced by the injection of adrenalin.

During the half hour following the termination of the 5-minute injection period the decline of the blood sugar level was more rapid in the glucose than the adrenalin series (fig. 1); at the time intervals, 5, 15 and 30 minutes after injection, blood sugar following glucose injection was 58, 47 and 11 mg. per cent above normal; the corresponding values after adrenalin are 75, 57 and 51 mg. per cent. Whatever the cause of this difference, it cannot be a reduced uptake of sugar by the peripheral tissues following adrenalin since it will be shown below that, if anything, this is then greater than after pure glucose administration. One thinks of possible inhibition of insulin secretion by the injected adrenalin and a retarded reconversion of blood sugar to glycogen; but although such an inhibitory effect has been reported, a contrary action has also been claimed for it, so that the effect observed here will have to await explanation.

Sugar uptake. Without exception, arterial blood sugar level was always higher than that in venous blood leaving the hind leg; the average arterio-venous (A-V) difference for the 50 normals of this group of experiments was 21 mg. per cent; this represented an average rate of glucose retention by the leg tissues of 3.22 mg. per minute.

The adrenalin and glucose hyperglycemias (+77 and +82 mg. % respectively) were accompanied by an increase in this transfer of glucose from blood to leg tissues (fig. 1): at the end of the 5-minute injection of adrenalin, the A-V difference increased to 45 mg. per cent, and glucose retention to 7.07 mg. per minute, or an increase of the latter to 220 per cent of normal; the corresponding figures at the end of the 5-minute glucose injection are 31 mg. per cent, 6.05 mg. per minute and 188 per cent of normal. Thus, if anything, the slightly lesser adrenalin hyperglycemia is accompanied by a greater increase in the transfer of glucose to these peripheral leg tissues than is the slightly greater one resulting from glucose administration. Per-

haps there is nothing significant in this, but at least it demonstrates that adrenalin has no specific inhibitory effect on the uptake of blood sugar by the peripheral tissues during the development of its hyperglycemic effect.

This latter is also true for the most part during the half-hour post-injection period. For the intervals 5, 15 and 30 minutes after the end of injection the figures are: adrenalin series: A-V difference, 28, 21 and 24 mg. per cent; glucose uptake, 4.37,

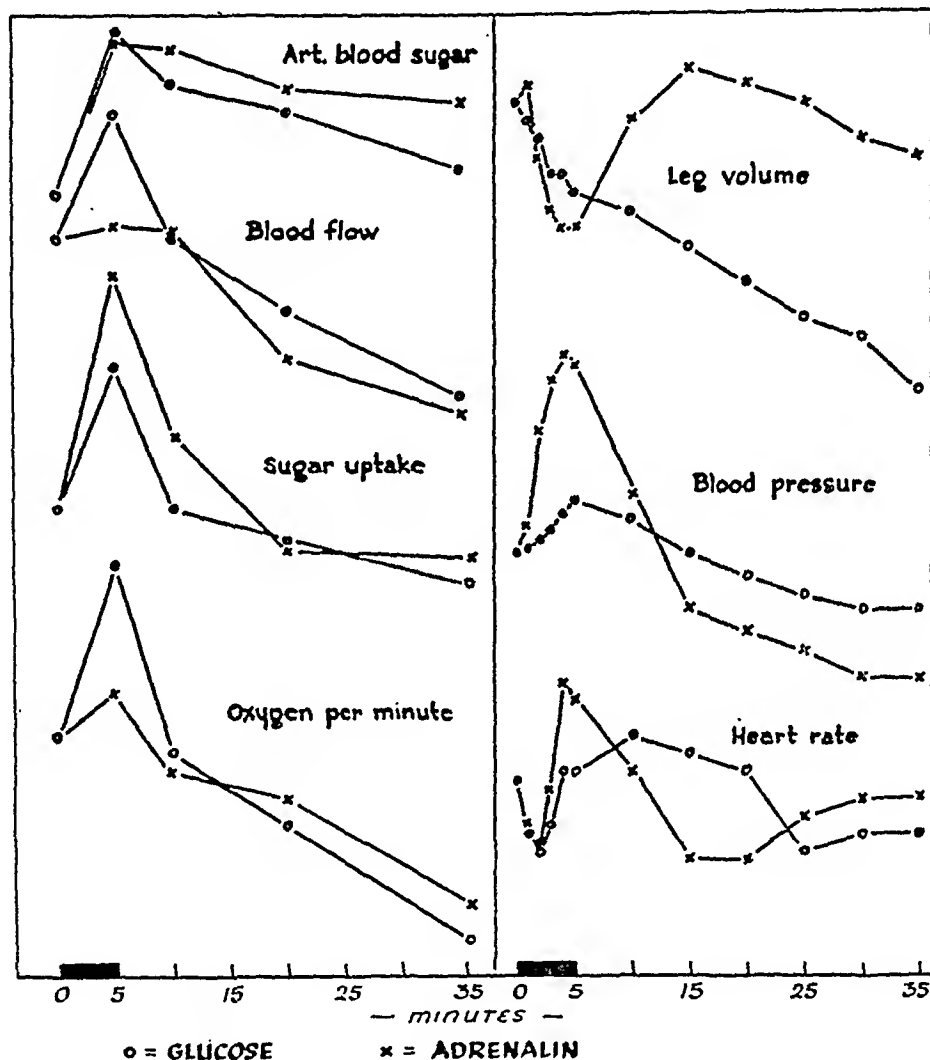


Fig. 1. EFFECT OF 5-MINUTE INTRAVENOUS INJECTION of adrenalin (0.004 mg/kg/min.) and of glucose (0.05 gm/kg/min.) on the circulation in and glucose retention and oxygen utilization by the hind leg tissues of dial-urethane (Ciba) anesthetized cats. For description see text.

2.33 and 2.26 mg. per min.; per cent of normal, 136, 72 and 70; glucose series: A-V difference, 22, 22 and 20 mg. per cent; glucose uptake, 3.37, 2.84 and 2.00 mg. per min.; per cent of normal, 105, 88 and 62. Thus, except for the interval 15 minutes after injection, the transfer of sugar from blood to leg tissues continues to be greater after adrenalin than after glucose administration (fig. 1).

During this 30-minute after-injection period the blood sugar level is declining, but more rapidly after glucose than after adrenalin injection; and this better-sustained arterial sugar concentration after adrenalin might be a factor in maintaining

the just-mentioned greater transfer from blood to tissues which continues during this half-hour post-adrenalin as compared with the post-glucose period. On the other hand it could have been anticipated that in both series as soon as the hyperglycemias began to decline from their highest levels, the transfer of glucose would reverse and be from tissue to blood. This would be particularly expected if the increased blood-to-tissue transfer at the height of the elevated blood sugar levels is regarded as merely a physical equilibrium involving no increase in glucose metabolism or utilization. Whether or not it is of any significance in this connection it may be remarked, however, that such a reversal was never seen during the post-injection periods of declining arterial blood sugar level; the A-V differences were always positive and the glucose transfer always from blood to tissue, even though the total transfer per minute had fallen, 15 and 30 minutes after injection, to only 88 and 62 per cent (glucose series) and 72 and 70 per cent (adrenalin series) of normal. This ultimate reduction to less than normal sugar uptake by the tissues may be linked with the greatly reduced blood flow which occurred at this same time.

Blood flow. The average rate of blood flow through the hind leg for the 50 normal determinations was 15.3 cc/min.; this agrees well with a previous average (1) of 16.4 cc/min. for 212 measurements of normal flow rate.

Adrenalin effect. At the end of the 5-minute intravenous injection of adrenalin at the rate of 0.004 mg/kg/min., flow rate was increased only 3 per cent for the average of the entire series of 25 experiments (fig. 1); this is approximately of the same magnitude as, though somewhat less than the increase of 8 per cent as the average of the 44 experiments of the previous work, just referred to, in which adrenalin was injected at this same rate.

The value of such confirmation resides chiefly in the fact that the effect on blood flow of adrenalin injected intravenously at the rate used here is, in individual instances, unpredictable; it may be increased or decreased. Further reassurance that the effects obtained are not entirely capricious but are following some pattern imposed by the experimental conditions, such perhaps as varying depth of anesthesia or other factor or factors as yet unsuspected, is derived from the relatively constant proportion of decreases to increases: in the present work these were 36 and 64, in the former, 43 and 57 per cent, respectively.

Some insight into the mechanism of this variability of response is afforded by the blood pressure, heart rate and leg plethysmograph records which were concurrently obtained and the averages of which for the entire series are shown in figure 1.

Comparing those experiments in which blood flow was increased, with those in which it was decreased at the termination of the 5-minute adrenalin injection period, it is found that the plethysmographic change, measured as the deviation in millimeters from the normal base-line, is practically the same in both groups. The change, as the average per cent of normal, for each minute of the 5-minute injection was for the 16 experiments in which blood flow was increased: 118, 65, 50, 42 and 41; and for the 9 experiments in which blood flow was decreased: 104, 76, 48, 45 and 57; thus, what difference there is in the average reaction of the two groups, indicates a slightly greater decrease of leg volume (vasoconstriction) when blood flow was increased.

The cause of this increased blood flow in spite of slightly greater reduction in

leg volume is probably found in the average change in arterial blood pressure of the two groups. The averages for each minute of the 5-minute injection period were, when blood flow increased: 8, 32, 46, 56 and 54 mm. Hg above normal; and when blood flow decreased: 3, 19, 25, 3 and 1 mm. Hg above normal; i.e., in the first group, blood pressure rises progressively during the first four minutes of injection and then steadies at a high level, 51 to 54 mm. Hg above normal; whereas, in the other, after reaching a maximum 25 mm. above normal during the third minute of injection it drops rapidly almost back to normal.

The behavior of heart rate is also interesting during this 5-minute injection period. The averages for each minute for the group in which blood flow was increased were, as deviations in beats per minute from normal: -2, -9, -6, +11 and +14; for the group in which blood flow decreased: -12, -6, +8, +9 and zero; i.e., in both groups there is an initial cardiac inhibition as the blood pressure rise gets under way but this is more alert and severe in the latter group; in this group, too, the secondary tendency to increase in rate is quickly and effectively checked so that the rate is finally held at normal.

In summary, two groups of effects during the last minute of a 5-minute intravenous injection of adrenalin are correlated as follows: in one group blood flow through the leg is increased; at this time arterial blood pressure is markedly elevated, heart rate is above normal and leg volume is decreased; in the other group blood flow in the leg is decreased, blood pressure and heart rate are either practically or entirely unchanged and there is slightly less decrease in leg volume.

Not knowing of any previous results of just this nature, or, therefore of attempts to explain them, an hypothesis may tentatively be advanced to account for them, or at least serve as a guide to further work. We will assume that the ultimate cardiac and smooth muscle responses, cardiac acceleration and peripheral vasoconstriction, are probably not markedly variable for a fixed rate of intravenous adrenalin administration, and probably not markedly affected by chance variations of the fairly fixed conditions of these experiments. It seems altogether probable, however, that the nervous regulatory mechanisms, such as the moderator reflexes, might be significantly affected in sensitivity by such variations in duration or intensity of narcosis as might inadvertently occur from time to time in the same animal or especially as between different preparations. If these were not depressed, any tendency of blood pressure to rise or heart rate to increase as a result of the peripheral action of adrenalin would be effectively countered by reflex cardiac and vasoconstrictor inhibition; the vasoconstrictor decrease in leg volume would be moderated and the cardiac acceleration and blood pressure rise, as a result of 'over-shooting', might for a time be completely abrogated. These are the conditions observed in the group of experiments in which as a consequence of the remaining peripheral vasoconstriction and the failure of arterial pressure to be elevated, the amount of blood driven through the leg per minute is diminished. On the other hand if the moderator reflex centers were sufficiently depressed, blood pressure and heart rate would increase with less effective counteraction and although peripheral vasoconstriction would remain even somewhat more intense than in the first group, the sizable elevation of pressure could drive even more blood through still patent channels so as to augment it even above normal.

This matter is dwelt upon at this length in a report whose chief interest is in the effect of adrenalin on tissue oxygen consumption because as will be seen below there is good evidence that at least in large part its effect on this is secondary to that on blood flow. It is also a useful corrective for what seems to be the general opinion that the effect of adrenalin is either one thing or another; our experience has been that at least in so far as peripheral blood flow and oxygen consumption are concerned the effect under the usual experimental conditions is qualitatively variable precisely, as we suppose, because of the complex underlying interrelationships just reviewed.

Glucose effect on blood flow. Glucose administered intravenously in the amount used here, 0.05 gm/kg/min. for 5 minutes, increased blood flow in the hind leg during the terminal part of the last minute of injection from the average normal of 15.3 to 19.5 cc. per minute or 28 per cent as the average of the entire series of 25 experiments (fig. 1). By contrast, it will be recalled that the corresponding total average for the effect of adrenalin was the very slight increase of only 3 per cent. Also in contrast to the effect of adrenalin, that of glucose was more constant in that flow rate was increased in 20 of the 25 experiments; of the remaining five there was practically no change in two and for the three in which there was an actual decrease the average of -2.5 cc/min. was much less than the average decrease of 7.9 cc/min. for the nine of the adrenalin series in which this occurred. It may be said, then, that the effect of glucose administered intravenously in the amount used here on blood flow in the tissues of the leg is predominantly to increase it and to a rather marked degree.

The mechanism of this increase can be deduced only in part from the concomitant records of blood pressure and leg volume (fig. 1). Such change in blood pressure as occurred would contribute to it, but perhaps could not be considered its only cause for it can be seen that the average increase is only slight, +11 mm. Hg, as compared with the large increase of 44 mm. induced by adrenalin. As to why blood pressure should be increased at all, it is probable that the small amount of injected fluid, 0.5 cc/min. for 5 minutes, was without effect; nor, apparently, did the heart contribute to it, since after an initial depression of rate as the blood pressure rise was getting under way it returned only to the pre-existing normal as the injection ended.

It is seen however that during the 5-minute injection period, leg volume is progressively decreased and to almost as great an extent as by adrenalin. It is unlikely that with glucose this decrease would be due to vasoconstriction. In fact, the contrary is suggested by the evidently active moderator reflex and cardiac inhibition which obtains at least during most of the injection period. If there were a corresponding reflex constrictor antagonism of any degree or even if there were no vasomotor effect in the leg at all, the decrease in leg volume would have to be due to osmotic abstraction of water into the hypertonic, sugar-enriched blood. This seems theoretically likely and would explain the rise in arterial blood pressure as a result of hydremic plethora. In fact, in spite of contrary evidence this is the only mechanism we can think of; but it must be recalled in this connection, as mentioned in a previous section on blood hydration, that all blood hemoglobin determinations indicated passage of water from blood to tissues; i.e., venous blood from the leg always had a higher hemoglobin concentration than the arterial blood going to it; and this trend was never reversed, but only reduced slightly below the normal value

by injection of glucose. It is therefore impossible from these data to account satisfactorily for the marked and almost unexceptional increase of blood flow in the leg which resulted from the injection of glucose; an alternative explanation based on the local vasodilator action of increased tissue metabolic rate will be more appropriately presented in a later section in connection with the effects on oxygen consumption by the leg.

After effects. It is seen (fig. 1) that five minutes after the injections were ended blood flow in both the adrenalin and glucose series had returned to the pre-existing normal value. Thereafter decline is continuous and almost uniform but slightly faster in the adrenalin series to final values approximately 60 per cent of normal 30 minutes after injection. This is similar to the decline we have always observed during observation periods of this duration even in controls without any injection whatever and is probably attributable merely to the deteriorating condition of the animal as a result of the frequent blood sampling and other trauma. It can be seen that blood pressure shows a similar decline and heart rate is for the most part failing.

The most interesting event during this half-hour post-injection period is the contrasting behavior of leg volume in the adrenalin and glucose series. After adrenalin the volume returns to, and slightly above normal shortly after the injection has stopped and falls below normal only toward the end of the half-hour period. After glucose, on the other hand, the sharp initial decrease in volume during the injection is followed by a continued decline at a lesser but steady and unbroken rate until the end. If decrease of leg volume in this case is due to osmotic attraction of water from tissues to blood, this evidence would indicate a rather slower equilibrium than might have been anticipated; if it is not due to this (and see objections above) we have no explanation for it.

Blood pressure, heart rate and leg plethysmograph. The only interest these have for the present is the insight they might afford as to the mechanism of blood flow change in the leg. As such they have been used in the preceding section on *Blood flow* and will receive no further consideration here.

Oxygen utilization by the leg tissues. It may be recalled that the impetus behind this work was a previous observation that although glucose uptake by leg tissues was invariably largely increased during an adrenalin hyperglycemia, their oxygen utilization was not, but appeared to be related rather to the effect of adrenalin on the circulation in the leg. Since there was existing evidence that a carbohydrate plethora from intravenously administered glucose could by itself stimulate the oxygen utilization of peripheral tissues (specific dynamic action) it became desirable to know whether the increase in the amount of glucose getting into the tissues to serve as stimulus was less when the hyperglycemia owed its origin to adrenalin, than when it resulted from glucose administration alone; and also whether oxygen utilization by the leg tissues following glucose administration would have any relation to altered blood flow as after adrenalin.

The first of the above questions has been answered in a previous section where (and also see fig. 1) it was shown that from almost exactly equal hyperglycemic levels the sugar uptake by the leg tissues was actually greater (220% of normal) when the hyperglycemia was due to adrenalin than when due to glucose alone (188% of normal). Therefore from the point of view of tissue carbohydrate plethora and stimulus, alone,

glucose specific dynamic action should be greater in the adrenalin than in the glucose series.

It will now be seen that not this, but its reverse is true. For each group as a whole (average of 25 experiments each) oxygen consumption was increased at the end of the 5-minute intravenous injection of adrenalin only 5 per cent; at the end of the 5-minute intravenous glucose injection the increase is 19 per cent; i.e., the considerably greater increase in sugar uptake (adrenalin) is associated with a very small increase in oxygen consumption, whereas the lesser increase in tissue sugar retention after glucose administration is accompanied by a marked increase in oxygen utilization.

Correlation of oxygen utilization with blood flow, however, corroborates our previous observation and is, for the total averages, as follows: glucose hyperglycemia is accompanied by an increased leg blood flow of 28 per cent and leg oxygen consumption of 19 per cent; and for the adrenalin hyperglycemia the corresponding figures are 3 and 5 per cent, respectively.

It will be recalled that neither adrenalin nor glucose administration were invariable in their effects on leg blood flow. With adrenalin, flow rate was increased in 16 and decreased in 9 experiments; and for the glucose series the corresponding figures were 20 and 5, respectively. In all but one of the 16 in which adrenalin increased flow rate, oxygen utilization increased also; and in only 2 of the 9 in which flow rate decreased did oxygen consumption fail to decrease also. Likewise in the 20 experiments in which flow rate increased as a result of glucose administration, oxygen consumption failed to increase in only 5 and these were rather in the range of no significant change than of definite decreases; and in the 5 experiments in which flow rate decreased with glucose injection, oxygen utilization also decreased in 3, with, again, the 2 exceptions being rather in the class of no marked change than of definite increases.

This correlation between blood flow, rather than glucose uptake, and leg tissue oxygen consumption is further exemplified by the post-injection trends of these three variables; as shown graphically in figure 1 the rates of decline of flow rate and oxygen utilization are very similar in these deteriorating animals while the course of change in sugar uptake is quite different.

It seems necessary to conclude therefore that the failure of an adrenalin hyperglycemia to be accompanied more generally and to a greater degree by increased oxygen utilization by peripheral tissues than is hyperglycemia resulting from glucose injection, is not due to a reduction by adrenalin of the amount of stimulating blood sugar which reaches and is retained by the tissues. Rather, what seems determinant for oxygen consumption is blood flow. Both as a rule are decreased and increased together; and since the effect of glucose alone is to cause a much greater average increase in leg blood flow it is also accompanied by a much greater average increase in leg tissue oxygen utilization.

The actual mechanism underlying these correlations may tentatively be suggested as follows: increased influx of glucose into the tissues of the leg stimulates their respiratory metabolism, i.e., has its usual specific dynamic action. Increased carbon dioxide formation could cause local vasodilatation thus providing a major factor for the increased blood flow following glucose infusion as well as an increase in the oxygen supply to the tissues commensurate with their new metabolic state.

If adrenalin in the amount used here is present in the blood reaching the leg tissues, vasoconstriction of varying degree depending on local conditions may be safely presupposed at least for these quiescent anesthetized animals; the effect might be quite different under conditions of normal and especially of emergency activity. Arteriolar constriction could be sufficient to block the passage of red cells but still permit a flow of plasma of high glucose content, the plasma skimming observed by Hartman *et al.*, (8). Passage of glucose into the tissues might then be equal for the short-time period used here to that in the previous instance, but oxygen for its oxidation would not reach the tissues at the same time. Such would be the most extreme case; but since this local reaction in the leg would conceivably be of almost any degree of intensity and since arterial perfusion pressure following general intravenous administration may also be increased over wide limits the net result as has been seen will necessarily be variable. And the only generalization that could safely be made would be that blood flow and oxygen utilization under the influence of glucose alone would very likely be more generally increased and to a greater degree than when adrenalin is present at the same time. And this could be used as a statement of the main conclusion to be derived from this work.

It may be emphasized again that this conclusion is intended to apply only to the conditions of these experiments. If adrenalin were employed in minimally effective and vasodilator or maximally constrictor dosages or if instead of being quiescent, anesthetized and progressively moribund the animals had been in more normal physiological condition, the results might be expected, from generally accepted premises, to be quite different.

SUMMARY

Intravenous injection into dial-urethanized cats of adrenalin at the rate of 0.004 mg/kg/min. for 5 minutes and of glucose at the rate of 0.05 gm/kg/min. for 5 minutes produced hyperglycemias of marked average similarity: +77 and +82 mg. per cent respectively. From these hyperglycemic blood sugar levels glucose transfer from blood to leg tissues was increased 120 and 88 per cent by adrenalin and glucose, respectively; so that adrenalin at least did not inhibit this effect.

The average effect of adrenalin on blood flow through the leg was to increase it 3 per cent; of glucose, 28 per cent. Both might in individual instances cause either an increase or a decrease; but after glucose the increases were more common and of greater degree. Oxygen utilization by the leg tissues followed the change in blood flow rather than of glucose uptake; thus, glucose injection increased it 19 per cent and adrenalin injection only 5 per cent.

Records of arterial blood pressure, heart rate and leg volume (plethysmograph) were obtained and used in an effort to account for the observed changes in leg blood flow.

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THYROID ACTIVITY AND RESISTANCE TO HISTAMINE-INDUCED PEPTIC ULCER AND TO ACUTE HISTAMINE POISONING¹

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IT has been recognized for some time that a relationship exists between the functional state of the thyroid gland and the secretory and motor activity of the stomach. One of the early observations was made by Friis Moller in 1914 (1) who noted that anacidity was common in patients with hyperthyroid disease. The feeding of thyroid substance to dogs (2) and rabbits (3) has been reported to diminish gastric secretion. Gastric motor activity appears to be augmented in dogs fed thyroid substance (4) and this effect is not altered by vagotomy (5). Truesdell (2) working with Pavlov pouch dogs observed marked reduction of acid secretion after feeding desiccated thyroid and opined that some disturbance of the nervous control of gastric secretion had occurred. Several thyro-gastric relationships have been described in the clinical literature. Increased incidence of peptic ulcer in connection with hypothyroidism and the converse have been noted (1) as well as successful therapeutic use of desiccated thyroid in hyperacidity and peptic ulcer (6). On the other hand Friedenwald and Morrison state (7): "The gastric acidity is usually diminished in hypothyroidism more frequently than in hyperthyroidism."

It was decided to investigate the effect of alterations of thyroid activity upon the gastric response to histamine as measured by the experimental production of peptic ulcer.

METHODS

Seventy-five male guinea pigs were used in this investigation. A commercial ration, fortified with ascorbic acid, and water were allowed *ad libitum* and the animals were kept in a room maintained at $27^{\circ} \pm 1^{\circ}\text{C}$.

Basal metabolic rates are expressed as Cal/hr/kg. $\frac{3}{4}$ and were determined in a modified Haldane apparatus (8) just prior to histamine treatment.

Since the metabolic rates were determined on groups of 6 animals, the presence of an atypical individual, e.g. an animal with incomplete thyroidectomy, might be reflected as a small error in the group average. Body weight records revealed, however, that all animals reacted, grossly at least, in a manner typical of the altered thyroid function.

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Histamine was administered intramuscularly in the form of a suspension in beeswax and mineral oil³ (9, 10). It was given in two equally divided doses totaling 1 mg/100 gm. of body weight per 24 hours. When benadryl (beta dimethylamino-ethyl-benz-hydryl ether hydrochloride) was used to protect the animals against the acute toxic effects of histamine, it was administered intraperitoneally, 30 minutes before each administration of the histamine suspension, in a dosage of 1 mg/100 gm. of body weight. This dosage of benadryl has been shown to be more than adequate to protect guinea pigs against 5 or 6 times the lethal dose₁₀₀ of histamine administered intravenously (11).

Natural crystalline thyroxine (Squibb) was given in a dosage of 0.1 mg. subcutaneously every other day. Desiccated thyroid was given orally in doses of 30 mg. daily and carefully washed down with water. Thiouracil was given *ad libitum* in the form of a 0.1 per cent solution in place of drinking water. Thyroidectomy was performed under ether anesthesia and the gland totally extirpated with preservation of the parathyroids. The animals were permitted a six-week recovery period before the B.M.R. was measured and histamine treatment begun.

In all cases medication was continued during the histamine administration. The end point of the experiment was death, due to either perforation of a peptic ulcer with consequent general peritonitis or acute histamine poisoning. Those animals failing to expire after 21 days of histamine administration and which, on post-mortem examination, presented no evidence of peptic ulcer were considered 'histamine resistant'.

Time was measured in hours from the first histamine injection to the time of death. All animals were autopsied and microscopic examination made of the organs of animals of each group. During the course of the work the animals were examined at such intervals that a maximum delay of four hours in noting death was possible but, in general, it was less than two hours.

Various experimental procedures were carried out on groups of animals as follows:

Group I: 9 animals. Average body weight 460 gm. Basal metabolic rate was measured and the histamine injections begun.

Group II: 8 animals. Average body weight 451 gm. These animals were treated like those of *Group I* except that benadryl was administered 30 minutes before each injection of histamine suspension.

Group III: 11 animals. Average body weight 490 gm. These animals were thyroidectomized, allowed a 6-week recovery period and then treated like *Group I*.

Group IV: 11 animals. Average body weight 484 gm. These animals were premedicated for 6 weeks with thiouracil and then treated like *Group I*.

Group V: 12 animals. Average body weight 504 gm. Natural crystalline thyroxine as described was given for 6 weeks before histamine injections were begun.

Group VI: 12 animals. Average body weight 440 gm. This group was treated like *Group V* except that benadryl protection was given as described for *Group II*.

Group VII: 6 animals. Average body weight 430 gm. These animals were given desiccated thyroid as described for 6 weeks before histamine injections were begun.

Group VIII: 6 animals. Average body weight 510 gm. These animals, too, were given desiccated thyroid and treated like those of *Group VII* but in addition were given benadryl protection as described.

³ Histamine-Beeswax was a gift from Hoffman-LaRoche, Inc., Nutley, N. J.

RESULTS

Table 1 summarizes the results obtained with *Groups I* to *IV*. *Groups V* and *VII* were both made hyperthyroid and may be considered jointly. The metabolic rates for *Group V* (thyroxin) and *Group VII* (desiccated thyroid) were 31 per cent and 17 per cent, respectively, above the value for *Group I*. Every animal in both groups died within half an hour after the first histamine injection. These animals in all cases exhibited the typical manifestations of acute histamine poisoning and, of course, no evidence of peptic ulcer. This response to the suspension of histamine, which produced no acute reactions in most normal and hypothyroid animals, was so dramatic and definite that it was decided to attempt protection of later hyperthyroid groups with benadryl.

Groups VI and *VIII* may also be considered together. These hyperthyroid animals were given benadryl in the hope that fatal reactions to histamine might be prevented and thus permit the usual observation of the development of perforated peptic ulcers. The survival time was increased but the animals, nevertheless, died before the typical ulcer endpoint was reached. All of the animals succumbed to acute histamine poisoning shortly after one of the first seven injections of histamine, i.e. within 74 hours. Of the 18 animals in *Groups VI* and *VIII*, 9 died within 12 hours after histamine dosage was begun, 5 more died in the next 48 hours and the last 4 animals died at 74 hours. Among the 4 succumbing to the seventh injection only two had nonperforated peptic ulcers. The remaining 16 animals failed to exhibit any signs of ulcer.

STATISTICAL ANALYSIS

Application of the 't' test and 'Student's' chart to the data on survival times yields the following comparisons among the first four groups:

GROUP	IV	III	II
<i>I</i>	t = 0.79 p = 0.45	t = 3.43 p = 0.004	t = 0.706 p = 0.48
<i>II</i>	t = 0.044 p = 0.97	t = 2.251 p = 0.040	
<i>III</i>	t = 2.32 p = 0.033		

Thus there are significant differences between groups *I* and *III*, *II* and *III* and *III* and *IV*. Since the animals in *Groups V*, *VI*, *VII*, and *VIII* died rather promptly of acute histamine poisoning they did not lend themselves to a similar analysis.

TISSUE EXAMINATION⁴

All of the animals that died of perforated ulcer with peritonitis—*Groups I*, *II*, *III*, *IV*—showed the familiar pathology of an acute and fulminating type of peptic ulcer of the stomach or duodenum, with no discernible gross or histological differences among specimens from the different groups.

The animals that died of acute histamine poisoning—*Groups V*, *VI*, *VII*, *VIII*—

⁴ The writers are indebted to Dr. Wm. B. Hawkins, of the Department of Pathology, for confirmation of the tissue examinations.

also showed uniform changes among themselves, consisting of pulmonary hemorrhages, areas of pulmonary atelectasis and meningeal hemorrhages. There were no discernible gross or histological differences among specimens from the different groups. The pathology seen in these animals also did not differ from that seen in control animals not pre-treated with thyroid substance which were killed by an overdose of histamine for purposes of comparison. The animals treated with thiouracil showed the characteristic goitrous changes.

DISCUSSION

Thyroidectomy obviously accelerates the rate of development of histamine-induced peptic ulcer. The data presented do not permit the conclusion that the perforated ulcers were caused only by excess gastric acid secretion but it seems likely that

TABLE I. EFFECT OF THYROID ACTIVITY ON HISTAMINE-INDUCED PEPTIC ULCER

GROUP	PRE-HISTAMINE TREATMENT	BMR, CAL/HR/EG ^{2/4}	MEAN SURVIVAL TIME, HRS.	CAUSE OF DEATH	REMARKS
I (9) ¹	None	3.38	161 ± 35 ²	Perforated peptic ulcer with peritonitis	2 survivors, histamine-resistant, no ulcer ³
II (8)	Benadryl		123 ± 35	Perforated peptic ulcer with peritonitis	1 survivor, histamine-resistant, no ulcer ³
III (11)	Thyroidectomy	2.94	56 ± 8	Perforated peptic ulcer with peritonitis	1 survivor, histamine-resistant, no ulcer ³
IV (11)	Thiouracil	2.85	125 ± 29	Perforated peptic ulcer with peritonitis	1 survivor, histamine-resistant, no ulcer ³

¹ Number of animals. ² Standard Error. ³ Histamine-resistant animals were not included in calculations of mean survival times.

it was an important factor. The failure of benadryl to influence the course of ulcer induction confirms the work of other workers (12).

A sharp contrast in survival times is demonstrated between the thyroidectomized animals and those pre-treated with thiouracil. In both groups the deprivation of thyroxine or its inactivation was reflected in the fall of metabolic rate, gain in weight, decreased physical activity and growth of coat. These results suggest that the thyroid gland in addition to its role of energy metabolism regulator, possesses a function which is related to gastric secretion and possibly to resistance of the gastric mucosa to erosion. The data at hand do not permit a decision as to whether this function depends upon the anatomical integrity of the gland or upon its production of a specific substance other than thyroxine. It is interesting that thyroxine induces an increased secretion of intestinal juice which persists long after the effect on energy metabolism has subsided (13). From a consideration of the data now available it appears as if the thyroid gland exercises some sort of control over the 'acid-base balance' of the gastrointestinal tract.

No other account has been found of histamine sensitivity as a manifestation of the hyperthyroid state. One can only speculate on its relationship to the frequency of pneumonia and to the skin sensitivity seen in hyperthyroid subjects.

SUMMARY

The frequency, time of development, course, outcome and pathology of histamine-induced peptic ulcer in the guinea pig is unaffected by the administration of benadryl. Removal of the thyroid gland, with preservation of the parathyroids, reduces significantly the time required for perforation of histamine-induced peptic ulcer. Thiouracil in amounts adequate to produce depression of the B.M.R. and characteristic goitrous changes does not produce any significant variation from normal in frequency, development, course, outcome or pathology of histamine-induced peptic ulcer. The difference between this group and the thyroidectomized group is quite distinct. Guinea pigs made hyperthyroid by the administration of desiccated thyroid by mouth or crystalline thyroxine parenterally in amounts adequate to cause a rise in metabolic rate, loss of weight, hyperactivity and loss of hair, show a striking hypersensitivity to acute histamine poisoning. On this account it was impossible to investigate the effect of the hyperthyroid state on the formation, course or outcome of histamine-induced peptic ulcer.

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EFFECT OF DIET ON LIVER REGENERATION IN PARTIALLY HEPATECTOMIZED RATS

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IN A recent article Denton and Ivy (1) reported that a liver diet enhanced liver regeneration in partially hepatectomized rats. These investigators found that liver restoration was faster in a liver-fed series of rats than in a control series on stock diet (Purina Chow). The present study was undertaken to determine whether the increased rate of regeneration in Ivy and Denton's investigation was primarily due to the high protein content of liver or to a specific substance in the liver.

METHODS

White male albino rats weighing from 225 to 325 gm. were used. All the rats were pre-fed a specific diet for 3 days prior to surgery. On the fourth day the animals were weighed and surgery was performed. With the aid of ether anesthesia the anterior abdominal wall was shaved and a mid-line incision was made. The left lateral and median lobes of the liver were isolated and freed from their ligamental attachments to the stomach and diaphragm. A small hemostat was placed upon the branches of the hepatic vein and artery supplying these two lobes. The pedicle of these lobes was ligated and transected and the abdominal wall closed with two layers of black silk. The removed segment of liver was weighed moist after surgery and then placed in an oven at 100°C. The liver was desiccated for a period of 3 days or until a constant dry weight was obtained. Following surgery all animals were again placed on the same diet they were fed prior to surgery. Feeding was *ad libitum*. On the 11th day post-operatively all animals were killed. The body weight was recorded, the regenerated liver removed, weighed moist and then placed into the oven to be desiccated.

The 5 diets used were as follows:

Diet 1. 60 per cent casein synthetic diet.

Diet 2. 60 per cent powdered whole liver diet. The whole powdered liver used in this diet was manufactured by VioBin Corporation, Monticello, Illinois. This product is a whole, defatted dry hog liver, processed at 37°C.

Diet 3. 60 per cent casein diet with Lilly's 343 Liver Extract. The amount of '343' to be added to the balanced diet was calculated from the average amount of coagulated liver consumed by a 250 to 300-gm. rat each day. This was about 11 gm/day. Each gm. of raw hog's liver yields 0.0425 gm. of '343' liver extract. Therefore, in our diet for every 10 gm. of the 60 per cent casein diet 0.425 gm. of liver extract was added.

Diet 4. Coagulated beef heart. Minced fresh beef heart boiled in a small quantity of water for 2 to 3 minutes.

Diet 5. Coagulated liver diet. This consisted of fresh coagulated hog's liver. The liver was diced and submerged in boiling water for a period of 2 or 3 minutes. We found it necessary to use coagulated liver because fresh liver when allowed to stand in the animal cages for a period of 5 to 6 hours putrefied at such a fast rate that the animal refused to eat it after a short period.

Our synthetic diet (*Diets 1, 2 and 3*) included the following substances: dextrose, 17 per cent; cod liver oil, 3 per cent; brewer's powdered yeast, 8 per cent; U. S. P. salt mixture, 5 per cent; commercial lard, 6 per cent; and protein (casein or powdered liver) 60 per cent.

In computing the data, rats that lost over 10 per cent of their total body weight during the 11-day period from the time of surgery to time of death were not included in the statistical analysis.

The rate of liver regeneration has been computed in three different ways: 1) the ratio of liver weight to body weight at necropsy; 2) absolute increment in liver weight during the 11-day postoperative period; and 3) increment in liver weight relative to the amount of liver left in at the time of surgery. All computations were made on the basis of dry liver weight. The moisture content of the liver showed no significant variation in any of the groups studied.

The weight of the liver left in at the time of surgery was calculated by multiplying the liver weight removed (dried) by the factor 0.46. This factor is derived from the fact that on the average the left lateral and median lobes comprise 68.5 per cent of the total liver mass. The accuracy of this percentage has been checked by several investigators including Higgins and Anderson (2) and Denton and Ivy (1). Thus, if 68.5 per cent of the liver (left lateral and median lobes) is removed, 31.5 per cent of liver mass remains in the living animal, giving $\frac{31.5\%}{68.5\%} = 0.46$ (factor). From the liver weight at the time of death is subtracted the liver weight left in at the time of surgery to give the value called the absolute increment.

Relative increment is calculated by dividing the increase in liver weight between the time of partial hepatectomy and the time of death (11 days later) by the liver left in at the time of surgery. Thus, the relative increment is equal to the number of gm. of liver regenerated per gm. of liver left in at the time of surgery.

RESULTS AND DISCUSSION

The results are summarized in table 1. It will be noted that the coefficients of variation in each group are smaller for liver weight/body weight ratio (LW/BW) than for the absolute or relative increment. The absolute increment does not take body weight into account. The relative increment takes body weight into account indirectly by relating the increment to the weight of the liver left at the time of hepatectomy. However, we have calculated for all 5 groups the correlation coefficients for the relation of weight of liver left at time of hepatectomy to magnitude of increment and found no evidence of a correlation between these values (the correlation coefficients were: +0.11, +0.15, -0.29, +0.32, -0.43). The validity of the use of the liver weight/body weight ratio is supported by our failure to find a significant correlation between this ratio and the body weight itself, indicating that the value of the ratio is independent of the body weight within the range of weights

studied here. The correlation coefficients of the relation of body weight to the ratio LW/BW for the 5 groups were: -0.19 , -0.60 , -0.55 , -0.82 , and -0.25 .

TABLE 1. RATE OF LIVER REGENERATION IN RATS ON VARIOUS DIETS

GROUP NO. AND DIET	NO. OF RATS	BODY WT. AT TIME OF HEPATECTOMY	ABSOLUTE INCREMENT IN DRY WT. OF LIVER	RELATIVE INCREMENT IN DRY WT. OF LIVER	LW/BW RATIO AT NECROPSY ($\times 10$)
1. 60% casein	9	273 ± 6.8 C = 7.0%	1.74 ± 0.8 C = 12.6%	1.91 ± 1.0 C = 15.2%	101 ± 3.0 C = 8.4%
2. 60% powdered liver	16	288 ± 6.0 C = 8.3%	2.21 ± 0.08 C = 14.0%	2.59 ± 0.10 C = 16.2%	110 ± 2.9 C = 10.0%
Difference between groups 1 and 2		+15 t = 1.7	+0.47 t = 4.1**	+0.68 t = 4.6**	+9 t = 2.16*
3. 60% casein with liver extract	9	292 ± 8.3 C = 8.6%	1.37 ± 0.06 C = 14.6%	1.71 ± 0.06 C = 11.7%	81 ± 2.5 C = 8.6%
Difference between groups 1 and 3		+19 t = 1.8	-0.37 t = 3.7**	-0.20 t = 1.7	-20 t = 5.2**
4. Whole beef heart	6	314 ± 2.9 C = 2.2%	1.71 ± 0.06 C = 8.8%	1.71 ± 0.13 C = 18.1%	89 ± 2.4 C = 6.6%
Difference between groups 1 and 4		+41 t = 5.3**	-0.03 t = 0.3	-0.20 t = 1.3	-12 t = 3.0**
5. Whole liver	13	293 ± 7.0 C = 8.2%	2.15 ± 0.07 C = 11.2%	2.28 ± 0.10 C = 16.7%	112 ± 2.2 C = 7.3%
Difference between groups 4 and 5		+21 t = 2.1*	+0.44 t = 4.0**	+0.57 t = 3.2**	+23 t = 6.2**

The values in this table are mean values with their respective standard errors. C = coefficient of variation ($C = \frac{s}{\bar{x}} \times 100$) where s is the standard deviation of the distribution and \bar{x} is the

mean; t values have been calculated from the formula $t = \frac{\bar{x}}{\sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2) S_x^2}}}$ where \bar{x} is the difference between the two means being compared, n_1 is the number of items in the first group and n_2 the number in the second group and S_x^2 is the pooled sum of the squares of deviations of the items from their respective means.

A single asterisk after a t value indicates that the value is statistically significant at the 5% level of probability, a double asterisk at the 1% level and no asterisk indicates that a difference as great or greater than the one observed would be expected to occur by chance due to random sampling error oftener than 5 times in 100 similar experiments.

While these values as a group do not indicate a significant correlation, the fact that they are all negative is suggestive and it is probable that if a wider range of weights was examined a significant negative correlation would result. This would suggest that the rats with lower body weight tend to regenerate liver faster.

Inasmuch as the differences which are of greatest interest are statistically significant by all the criteria for measuring regeneration, the relation of a single criterion is not critical in this study. However, the above analysis suggests that LW/BW may be useful for future studies, perhaps with a correction factor for body weight.

The results presented in table 1 confirm the findings of Denton and Ivy in regard to the enhancing action of whole fresh liver diet on liver regeneration. In addition they reveal that this same beneficial effect is exerted by powdered whole liver incorporated into a synthetic diet. In each case, a comparison with another diet of equally high protein content (whole beef heart or synthetic diet with casein) shows that the effect cannot be attributed to the high protein content of the diet. Denton and Ivy found crude liver extract parenterally to be ineffective in promoting liver regeneration. Our studies show that crude liver extract is ineffective also when given orally. In fact, it tended to inhibit regeneration. As in the studies of Denton and Ivy (1), no gross or microscopic evidence of fattiness of the liver was present with any of these diets.

SUMMARY

In partially hepatectomized rats on a balanced synthetic diet, liver regeneration was more rapid in the animals fed a diet containing powdered whole liver as the source of protein than in those on casein. Similarly, freshly cooked whole pig's liver was superior to freshly cooked whole beef heart in supporting liver regeneration. A supplement of crude liver extract in the diet failed to enhance liver regeneration.

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CONTROL OF LIVER REGENERATION AND NUCLEIC ACID CONTENT BY THE THYROID, WITH OBSERVATIONS ON THE EFFECTS OF PYRIMIDINES¹

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IT WAS stated by Higgins (1) that feeding desiccated thyroid enhances the regeneration of livers in rats after removal of 75 per cent of the organ. The administration of thyroid was not begun until the day of operation, and no effects were noted until 7 to 14 days afterward, the liver being 20 per cent larger than in the control group at the end of 4 weeks. As Fogelman and Ivy (2) point out, however, liver regeneration is normally practically complete before the observed differences occur, hence it is doubtful if this can be called an effect on regeneration. It is, of course, true that an effect on liver size was demonstrated, and this is confirmed by the fact that unoperated animals under thyroid medication also showed liver hypertrophy. A similar effect induced by thyroxin injection upon the size of normal liver was observed by Sternheimer (3). The opposite condition—hypothyroidism—was investigated by Drabkin (4) who found that in the thyroidectomized rat liver regeneration, 14 days after lobectomy, was of smaller magnitude than normal, but “was not strikingly interfered with.”

The results which we are here reporting show that thyroid medication significantly enhances, and that (at least in young animals) thyroidectomy significantly depresses liver regeneration. In view of the goitrogenic action of certain thiopyrimidines the effect of administering these was also investigated, another purpose in doing so being the relation of pyrimidines to the nucleic acids, and the possible competitive inhibition of these by the thiopyrimidines. It was found that pyrimidines have no effect, but that thiopyrimidines are capable of depressing regeneration; and that the latter effect is not due to suppression of thyroid hormone formation.

METHODS

Inbred rats of the Wistar strain, varying in age from 2 to 6 months, according to the nature of the experiment, as indicated later, were used. Sex appeared to have no influence on the results. Partial hepatectomy was performed by removing the left lateral and median lobes. It was first noted by Higgins and Anderson (5) in 1931, and amply confirmed, that this removes with sufficient constancy about two-thirds of the liver. The amount of regeneration can be variously calculated; we have used the following formula: $\% \text{ regeneration} = \frac{\text{wt. of liver at autopsy} - \frac{1}{2} \text{ amount removed}}{\text{amount removed}} \times 100.$

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This represents, in percentage, approximately the amount of removed liver replaced by new tissue.

All surviving animals were killed 96 hours after lobectomy, this being chosen because at this time the most rapid phase of regeneration has been completed. The standard diet consisted of 25 per cent casein, 50 per cent sucrose, 20 per cent Crisco, 5 per cent salt mix (Phillips and Hart, 1935); Vipenta, 0.5 ml/kg. There is evidence of a somewhat conflicting nature that diet affects liver regeneration, and it was therefore necessary to avoid this variable. The experimental animals were not 'pair fed' with their controls, but the amount eaten by each animal was noted, and the diet of the control group held down to approximately the same level. We have not included the evidence in tables, but wish to say that there was no significant statistical deviation in the amounts of food eaten by the control and experimental groups, and that the changes of weight in the animals were also of the same approximate order. We therefore are confident that the results are not affected by these particular variables.

Care was taken that any group of experimental animals should be of similar age as their controls, since animals before maturity (i.e. younger than 3-4 months) regenerate liver more rapidly than older animals. This will appear later.

Thyroid was administered in the form of the desiccated gland, mixed with the food when given in large amounts (500 mg/day), introduced directly into the back of the mouth when given in small amounts (3.5 mg/day). After some experimentation the pyrimidines and thiopyrimidines were administered as 1 per cent by weight of the diet, mixed with the food. All of these substances were given for either 10 or 20 days before hepatectomy (as hereinafter indicated) as well as during the four-day period of regeneration.

Nucleic acids were determined according to the method of Schmidt and Thannhauser (6) and expressed in terms of phosphorus.

RESULTS

Effects of Thyroidectomy and Thyroid Administration. In table 1, it will be seen that in 4- to 6-month-old rats the regeneration 96 hours after lobectomy on a restricted diet is 62 ± 2.7 per cent (on an *ad. lib.* diet this would in animals of this age be considerably higher). In animals thyroidectomized six weeks earlier, though slightly lower, it is not significantly different. When dried thyroid is given, however, in amounts that are sufficient to produce definite hyperthyroidism (500 mg. dried thyroid, per rat of 200-300 gm/day for 10 days), there is a significant increase in the rate of regeneration, amounting to 77 per cent at the end of the four-day period. In thyroidectomized animals given this amount of thyroid, the chemical picture was similar to that of the normal animal which had been made hyperthyroid by medication; but none of the 7 animals studied survived partial hepatectomy.

The ribonucleic acid fraction (PNA) is somewhat diminished in the pre-lobectomized liver six weeks after thyroidectomy, but is not affected in the regenerated liver; the desoxyribonucleic acid (DNA) fraction is unchanged. When the normal animal is given large amounts of thyroid, however, there is a considerable increase in the nucleic acids, especially the PNA fraction, in both the pre-lobectomized and regenerating liver.

After thyroidectomy, the water content of the regenerating liver is slightly reduced, as is the nitrogen content. On the other hand, thyroid administration, while it has no effect on the water content, significantly increases the percentage of nitrogen in both the pre-lobectomized and regenerating organ. This is in accord with the observations of Sternheimer (*loc cit.*) on the protein content of *normal* liver after thyroxin injection. On the whole, then, in this age group thyroidectomy

induces somewhat equivocal results, while after heavy dosage of thyroid, effects occur indicating increased capacity for regeneration, as well as mobilization of proteins, including nucleoproteins.

TABLE 1. RELATION OF THYROID AND THIOPYRIMIDINE EFFECTS (4- TO 6-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER					REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt. Wet wt.	Total N	Nucleic acids			Dry wt. Wet wt.	Total N	Regen- eration
	PNA	DNA	Total			PNA	DNA	Total			
	mg. P/100-gm. wet wt.				%	mg. P/100-gm. wet wt.				%	%
Controls (34) ¹ ..	98 ±1.2	21 ±0.4	119 ±1.3	30 ±0.2	3.13 ±.027	109 ±1.5	21 ±0.1	130 ±1.5	30 ±0.1	2.97 ±.012	62 ±2.7
Thyroidectom- ized (21, 18) ..	89 ±2.9	20 ±2.3	109 ±3.6	30 ±0.2	3.06 ±.084	108 ±2.5	20 ±1.6	128 ±3.0	26 ±0.4	2.77 ±.061	56 ±4.7
Thiouracil, 20 days (12, 9)...	102 ±2.3	20 ±0.5	122 ±2.3	30 ±0.9	2.90 ±.109	107 ±1.9	19 ±0.8	126 ±2.1	26 ±0.7	2.67 ±.111	49 ±2.6
Thyroidectom- ized + thi- ouracil, 20 days (11, 3)...	87 ±1.1	18 ±0.27	105 ±1.1	28 ±0.5	3.05 ±.085	108 ±1.0	18 ±0.4	126 ±1.3	28 ±0.4	2.95 ±.068	58 ±7.6
Dried thyroid, 500 mg/day, 10 days (22, 16).....	122 ±1.0	26 ±1.9	148 ±2.1	29 ±0.1	3.49 ±.005	129 ±1.7	24 ±1.4	153 ±2.2	28 ±0.4	3.24 ±.006	77 ±3.6
Thyroidectom- ized + dried thyroid, 500 mg/day, 10 days (8, 7)....	139 ±1.8	28 ±0.7	167 ±1.9	28 ±0.3	3.36 ±.113	All died					
Thiouracil + dried thyroid, 500 mg/day 10 days (13)...	137 ±1.1	28 ±1.1	165 ±1.6	28 ±0.5	3.35 ±.080	146 ±1.8	25 ±0.9	171 ±2.1	26 ±0.3	3.42 ±.083	72 ±4.6
20 days (6)....	133 ±1.5	26 ±2.1	159 ±2.6	28 ±0.3	3.44 ±.065	All died					

¹ Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean = $\sqrt{\frac{\sum d^2}{n(n-1)}}$

These results are to be compared with experiments shown in table 2. In this case, the animals were younger, being approximately 8 to 9 weeks old at partial hepatectomy. The diet of the control group was again limited to approximately that of the thyroidectomized animals, as were the thyroid-fed. In this group the regen-

eration of the 'normal' animals was higher than in the older group of similar animals, amounting to 99 per cent at the end of 96 hours. The thyroidectomized groups, how-

TABLE 2. RELATION OF THYROID AND THIOPYRIMIDINE EFFECTS (2-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER					REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt. Wet wt.	Total N	Nucleic acids			Dry wt. Wet wt.	Total N	Regen- eration
	PNA	DNA	Total			PNA	DNA	Total			
	mg. P/100-gm. wet wt.				%	mg. P/100-gm. wet wt.				%	%
Controls (13) ¹ ...	97 ±3.5	24 ±1.8	121 ±3.9	30 ±0.3	2.94 ±.058	107 ±3.9	27 ±1.7	134 ±4.2	29 ±0.6	2.88 ±.085	99 ±7.7
Thyroidect. Without thi- ouracil, 20 days (11) ¹ .	82 ±1.5	19 ±0.6	101 ±1.6	29 ±0.3	2.80 ±.077	97 ±1.4	21 ±0.6	118 ±1.5	25 ±0.4	2.60 ±.081	56 ±2.0
With 1% thi- ouracil, 20 days (21, 15) ¹	96 ±2.6	20 ±0.5	116 ±2.7	30 ±0.3	2.89 ±.069	126 ±2.5	20 ±0.5	146 ±2.6	27 ±0.5	2.81 ±.028	61 ±3.3
Thyroidect. with 3.4 mg. dried thy- roid daily Without thi- ouracil 10 days (12, 10) ¹	111 ±2.1	24 ±0.8	135 ±2.2	31 ±2.2	3.00 ±.079	114 ±1.6	21 ±2.4	135 ±2.9	27 ±0.5	3.06 ±.211	97 ±7.8
30 days (10, 9) ¹ ...	113 ±2.9	25 ±1.1	138 ±3.1	29 ±0.3	2.79 ±.047	130 ±2.9	26 ±0.7	156 ±3.0	25 ±0.4	2.74 ±.064	82 ±6.9
With 1% thi- ouracil 10 days (13, 9) ¹	112 ±2.7	21 ±1.0	133 ±2.9	29 ±0.2	3.05 ±.071	121 ±1.4	23 ±0.8	144 ±1.6	28 ±0.4	2.97 ±.133	68 ±6.2
20 days (22, 11) ¹	112 ±1.3	24 ±0.5	136 ±1.4	30 ±0.3	3.24 ±.061	129 ±3.5	24 ±1.1	153 ±3.6	27 ±0.5	2.99 ±.075	66 ±4.7

¹ Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean = $\sqrt{\frac{\sum d^2}{n(n-1)}}$.

ever, whose thyroids had been removed 20 days before hepatectomy, regenerated only to the extent of 56 per cent, or no better than the older thyroidectomized ani-

mals and unmistakably less than the controls. There was also a reduction in the nucleic acids, both PNA and DNA, in both the pre-lobectomized and regenerating liver, as well as a tendency towards a decrease in the total nitrogen. The reason for diminishing the interval between thyroidectomy and hepatectomy to 20 days, instead of 6 weeks as in the group previously discussed, was to reduce the age level of the group for comparison with a similar age level in controls.

Another group of animals of this age level was, immediately after thyroidectomy, put on a replacement thyroid therapy. The intent, in this case, was not, as previously, to create a hyperthyroid animal, but simply to restore the putative amount of normal thyroid secretion. From the data of Astwood and Dempsey (8), this was computed at 3.5 mg. of desiccated thyroid per day per rat for rats of this age and weight (about 150 gm.). Under these conditions, the regenerative capacity of the liver was restored to normal in animals where hepatectomy was performed 10 days after thyroidectomy, and who received replacement thyroid medication during this period and the period of regeneration. When hepatectomy was performed under similar conditions 20 days after thyroidectomy, the regeneration was almost as great (table 2, row 4).

The nucleic acids of the controls in this series was higher after lobectomy than in the older animals. After thyroidectomy, the fall in nucleic acids, particularly PNA, was evident, though not large. On the other hand, there was a definite increase when dried thyroid was administered, though not as much as in the earlier series, on massive doses of the hormone.

Effect of Pyrimidines. In view of the fact that pyrimidines constitute a part of the nucleic acid molecule, it was felt that administration of pyrimidines might affect the nucleic acid mobilization and possibly show a differential effect, since uracil is found in ribonucleic acid, and thymine in deoxyribonucleic acid. Cytosine is found in both, but we did not have any of this material at our disposal. After some experimentation, based on our experience with the thiopyrimidines, both uracil and thymine (Schwartz) were given as 1 per cent of the diet for period a of 10 to 20 days. It will be seen in table 3 that in respect to the things we were observing, these substances were entirely without effect.

Effect of Thiopyrimidines. Two-thiouracil, 5-methyl-thiouracil (thiothymine), and 6-propylthiouracil³ were given to groups of 4- to 6-month-old rats as 1 per cent of the diet. In this amount, fed over a period of 10 days before hepatectomy (as well as during regeneration), the substances caused small decreases in regeneration (table 4); when the administration was continued for 20 days before hepatectomy, the decrease became much more marked, and was, if anything, greater than in thyroidectomized animals of this age level. Nevertheless, except in the case where thiothymine is given for 20 days, there was practically no effect on the nucleic acid content. After thiothymine and propylthiouracil administration for 10 days, there was a considerable increase in the total nitrogen, apparently indicating protein mobilization, but on continuance of the medication for 20 days, the nitrogen returned to normal. The significance of this is not clear. There is no reason to doubt that

³ We are indebted to Dr. E. B. Astwood for the thiouracil and to Dr. Stanton E. Hardy of the Lederle Division of the American Cyanamid Company, for the propylthiouracil used in this work.

the protein mobilization would also have been observed after 10 days of thiouracil medication, but this experiment was not performed.

Relation of Thyroid to Thiouracil Effects. When thiouracil medication was superimposed upon thyroidectomy (table 1), we were unable to keep more than 3 out of 11 of the older animals alive after hepatectomy. In these there was no summation of effect, the results corresponding very closely to that of thyroidectomy alone. In young animals (2 months old), with a shorter interval after thyroidectomy, the results were somewhat different. When thiouracil was given to such animals, the regeneration was reduced as in thyroidectomy (table 2, row 3), but,

TABLE 3. EFFECT OF PYRIMIDINES (4- TO 6-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER					REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt. Wet wt.	Total N	Nucleic acids			Dry wt. Wet wt.	Total N	Regen- eration
	PNA	DNA	Total			PNA	DNA	Total			
	mg. P/100-gm. wet wt.				%	mg. P/100-gm. wet wt.				%	%
Controls (34) ¹ ...	98 ±1.2	21 ±0.4	119 ±1.3	30 ±0.2	3.13 ±0.027	109 ±1.5	21 ±0.1	130 ±1.5	30 ±0.1	2.97 ±0.012	62 ±2.7
Uracil 1% 10 days (4) ¹ ...	93 ±0.6	21 ±0.3	114 ±0.7	31 ±0.5	3.08 ±0.086	106 ±1.2	21 ±0.0	127 ±1.1	30 ±1.2	3.22 ±0.215	64 ±4.8
20 days (12) ¹ ...	95 ±3.4	19 ±1.1	114 ±3.5	30 ±0.3	3.01 ±0.068	105 ±2.4	21 ±0.9	126 ±2.6	30 ±0.7	2.81 ±0.131	62 ±4.7
Thymine 1% 10 days (24) ¹ ...	91 ±0.8	21 ±0.4	112 ±0.9	30 ±0.2	3.74 ±0.059	112 ±3.6	21 ±0.3	133 ±3.6	31 ±0.4	3.04 ±0.067	63 ±3.7
20 days (18) ¹ ...	92 ±1.8	22 ±0.4	114 ±3.4	30 ±0.3	3.07 ±0.057	110 ±2.4	22 ±0.4	132 ±2.4	30 ±0.5	2.92 ±0.055	57 ±1.2

¹ Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies.

$$\text{Deviation measure} = \text{Standard Deviation of mean} = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

surprisingly, there was, after partial hepatectomy, a rise in the ribonucleic acid fraction.

When, in normal animals, dried thyroid and thiouracil administration were combined the result seemed to depend on the amount of thyroid fed. With large amounts of thyroid (table 1), the result was practically as though no thiouracil had been given. This was explicable on the ground that the thyroid effect was dominant, and the conclusion was buttressed by the fact that when only replacement amounts of desiccated thyroid were given with thiouracil (table 2) the latter was able to combat the effect of the administered thyroid as to regeneration, the latter being reduced almost to the same level as that of the thyroidectomized animals. However, the typical increase of nucleic acids seen after thyroid medication was not nullified by thiouracil.

DISCUSSION

The evidence points to a control of liver regeneration as well as of nucleic acid and protein mobilization by the thyroid. On the other hand, the fact that the effect of thyroidectomy on regeneration is clearly seen only in younger animals seems to indicate that as the animal approaches the adult state, control of regeneration can be taken over by other forces in the body. The thyroid hormone stimulates regeneration by the liver, and it may be that the greater regenerative capacity of the organ

TABLE 4. EFFECT OF THIOPYRIMIDINES (4- TO 6-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER					REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt. Wet wt.	Total N	Nucleic acids			Dry wt. Wet wt.	Total N	Regen- eration
	PNA	DNA	Total			PNA	DNA	Total			
	mg. P/100-gm. wet wt.				%	mg. P/100-gm. wet wt.				%	%
Controls (34) ¹ ...	98 ±1.2	21 ±0.4	119 ±1.3	30 ±0.2	3.13 ±.027	108 ±1.5	21 ±0.1	130 ±1.5	30 ±0.1	2.97 ±.012	62 ±2.7
Thiouracil 1%, 20 days (12, 9).....	102 ±2.3	20 ±0.5	122 ±2.3	30 ±0.9	2.90 ±.109	107 ±1.9	19 ±0.8	126 ±2.1	26 ±0.7	2.67 ±.111	49 ±2.6
Propylthiouracil 10 days (6, 5).. 20 days (15, 11).....	105 ±3.0	21 ±0.0	126 ±3.0	29 ±0.4	3.55 ±.057	114 ±1.8	22 ±0.1	136 ±1.8	27 ±1.2	3.47 ±.116	53 ±3.4
	96 ±1.5	21 ±0.7	117 ±1.7	30 ±0.3	3.31 ±.039	112 ±1.2	20 ±0.4	132 ±1.3	27 ±0.4	2.87 ±.088	42 ±3.1
Thiothymine 1% 10 days (6, 5).. 20 days (24, 22).....	98 ±2.4	21 ±0.6	119 ±2.5	30 ±0.5	3.45 ±.113	107 ±2.8	21 ±0.3	128 ±2.8	30 ±1.3	3.34 ±.067	58 ±1.3
	87 ±1.1	17 ±0.6	104 ±1.3	30 ±0.2	3.06 ±.078	106 ±2.1	18 ±0.6	124 ±2.2	30 ±0.4	3.00 ±.071	45 ±2.9

¹ Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

in the younger animal is a function of greater thyroid activity. The sharp drop following thyroidectomy in such animals, to the level in the older thyroidectomized animals, seems to bear this out; and the fact that it goes no lower indicates that as in the adult, other controlling factors are at work, though not in this case compensatory. The thyroid hormone also stimulates mobilization of liver nucleic acids, especially ribonucleic acid, though desoxyribonucleic acid, whose concentration is notoriously difficult to change, is also affected to some extent. According to Novikoff and Potter (8), the ribonucleic acid content is greatest during the period of most active regenera-

tion. This would be in harmony with the thyroid effect on the latter. On the other hand, on the basis of effects we have noted in other investigations, it seems to us that the necessary association of active growth with nucleic acid mobilization must be taken with some reserve. We therefore record the facts in this case without prejudice or postulate. We must also record the apparent mobilization of total protein with the same reservation.

As stated earlier in this paper, we were impelled to test the effect of pyrimidines by two major considerations. In the first place, the thiopyrimidines are goitrogenic, and it is therefore reasonable to expect that they may abolish the thyroid hormone effect on liver by preventing its synthesis. In the second place, ingested pyrimidines, despite certain evidence to the contrary (cf. Plentl and Schoenheimer (9)), might influence, in a rapidly growing organ, the nucleic acid content, and if the latter were a limiting factor, the actual growth of that organ. Also, in this connection, it is possible that the thiopyrimidines might act either as sources of pyrimidines or as competitive inhibitors of any effects the latter might have.

The results that we obtained indicate that the ingestion of pyrimidines added to a synthetic diet containing none, does not affect the synthesis of liver nucleic acid or regeneration of the organ. This tends to corroborate, as far as it goes, the evidence of Plentl and Schoenheimer.

As for the thiopyrimidines, in the amount given, they inhibited liver regeneration. This is, of course, a large dose, much above the minimum goitrogenic effect, though according to Astwood (10) not incompatible with continued existence with symptoms referable only to hypothyroidism, even when continued over a long period of time. Fogelman and Ivy (loc cit.) have shown that when rats were given intraperitoneal injections of 8 mg. thiouracil per 100 gm. of body weight daily following partial hepatectomy, the rate of regeneration was increased. This might correspond roughly to the ingestion of 0.1 per cent in the diet, or much less than the amount we gave. In view of these and our own findings, therefore, it appears that the effect of thiouracil, at least, is a function of the amount given. The inhibition of regeneration seen in our experiments is not a function of the anorexia and concomitant failure to gain, or actual loss of weight of the animals, since the controls were arbitrarily subjected to the same conditions. Nor is it a function of the depression of thyroid hormone synthesis, because when the normal thyroid hormone secretion was replaced by ingestion in a thyroidectomized animal, and the regeneration restored to normal, the latter was still depressed by thiouracil. We are led to believe that the thiouracil effect, whether depressant as in our case, or stimulating, as in the case of Fogelman and Ivy, is at least in part an effect either directly on the liver itself or on some other control than that furnished by the thyroid.

We set out to determine, among other things, whether thiopyrimidines would antagonize, perhaps by competitive inhibition, a possible pyrimidine effect. As we have already stated, we were unable to demonstrate this inhibition because administered pyrimidines were without effect. It is, however, not ruled out, for while it may be that ingested pyrimidines are not incorporated into nucleic acids, the pyrimidines of the latter in such case may be considered as being synthesized from other fragments of metabolism, and if so, the postulated inhibition may still occur after

such synthesis has taken place. Admittedly, we have no evidence for or against this.

SUMMARY

Observations have been made concerning the influence of the thyroid, and of pyrimidine and thiopyrimidine administration, upon rat liver regeneration and nucleic acid and nitrogen content. The evidence shows that the thyroid is a regulator of liver regeneration, as well as of liver nucleic acid and protein mobilization. In animals over four months old, but not in younger animals, the control over regeneration can, in the absence of the thyroid, be taken over by something else. Ingested pyrimidines have no effect on liver regeneration, or on nucleic acid and nitrogen content. Ingested thiopyrimidines, in high concentration, reduce the regenerative capacity of the liver; and this effect is not due to inhibition of the formation of thyroid hormone. A postulated inhibition of pyrimidines by thiopyrimidines was not proved; neither was it ruled out.

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BRAIN AND PLASMA CATIONS AND EXPERIMENTAL SEIZURES IN NORMAL AND DESOXYCORTICOSTERONE-TREATED RATS¹

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ALTHOUGH clinical studies on the efficacy of desoxycorticosterone therapy in epilepsy have yielded contradictory results (1, 2), in a few cases a significant decrease in the incidence of grand mal seizures has been noted (2). This improvement in the clinical status of the patients was apparently due to a retention of Na in the body, and a loss of K (3). Spiegel (4) administered desoxycorticosterone to rats but failed to observe a change in the convulsive threshold to electrical stimulation. On the other hand, a growing body of evidence indicates that the brain excitability of rats may be altered in a predictable manner by changes in the electrolyte balance of the body. Swinyard (5) has found a positive correlation between extracellular sodium concentration and electroshock seizure threshold in experimentally hydrated rats, and recent work in this laboratory (6) has demonstrated a lowered electroshock seizure threshold associated with low plasma Na levels in adrenalectomized rats. Evidence has also been presented which indicates that the refractory period following a major convulsion may be correlated with elevated plasma Na (7). In view of these observations it seemed desirable to reexamine the effect of desoxycorticosterone on brain excitability and to investigate the effects of excess Na, K, Ca and Mg.

METHODS

Adult Sprague-Dawley male rats were used as experimental animals. Electroshock seizure thresholds (6, 8) and maximal seizure patterns (9) were determined by the standard methods of this laboratory.

Isotonic solutions of the salts used were injected intraperitoneally in the dosages indicated. After 1 hour, an interval found to be sufficient for the absorption of most of the fluid, the electroshock seizure thresholds were determined. The thresholds observed, expressed in milliamperes (mA), were compared with control observations made 6 hours earlier. Maximal seizure patterns were also investigated before and 1 hour after the injection of the salt solutions. They were checked at 30-minute intervals thereafter until the greatest change from normal was found.

Desoxycorticosterone acetate² (DCA) was administered by the subcutaneous implantation of six 15-mg. pellets per rat. The difference in weight of the pellets before implantation and after removal indicated that the mean amount absorbed was 1.6 mg/rat/day.

Samples of plasma and of brain were collected 23 to 31 days after the implantation of DCA pellets or 1 hour after the injection of salt solutions. They were prepared and analyzed for Na, K,

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Ca, Cl and water by the methods in use in this laboratory (6). Plasma samples were analyzed for Mg by the method of Kunkel, Pearson and Schweigert (10).

RESULTS

The effects on maximal seizure pattern produced by the intraperitoneal injection of NaCl, KCl, $MgCl_2$ and $CaCl_2$ solutions and of modified Ringer's solution (pH 7.4) are illustrated in figure 1. Each group of 3 columns shows the duration of the phases of maximal seizures in a single animal and is typical of the responses of 10 to 25 animals treated similarly. The first column shows the control pattern, the second shows the pattern at the peak action of the injected salt, and the third column indicates the recovery of the control pattern. The injection of Ringer's solution had no effect on the seizure pattern. The injection of either NaCl or KCl solution resulted in abolition of the tonic phase and prolongation of the clonic phase, whereas

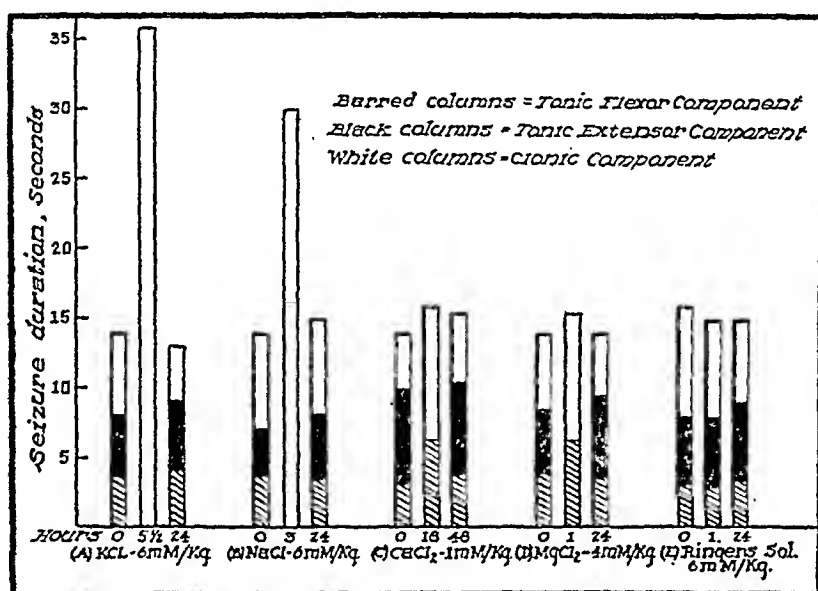


Fig. 1. EFFECTS OF INJECTED SALT SOLUTIONS ON THE MAXIMAL SEIZURE PATTERNS OF RATS.

the injection of $CaCl_2$ or $MgCl_2$ solution caused abolition of the tonic extensor component without change in the total duration of the seizure.

Table 1 presents the effects of the same salt solutions and of phosphate buffer on the electroshock seizure thresholds of normal rats. The injection of Ringer's solution again had no effect. Treatment with NaCl solution caused a 14 per cent rise in threshold, and $CaCl_2$ solution caused a smaller but significant rise. The injection of KCl, $MgCl_2$ or phosphate buffer resulted in a significant lowering of the threshold.

A summary of the observations made on the effect of DCA on the electroshock seizure thresholds of rats is presented in figure 2. The thresholds of 22 control and 22 treated animals were followed over a period of 31 days. The initial threshold of both groups was 20.5 mA. The controls showed no consistent change in threshold and on the final day had a threshold identical with that on the first day. The rats implanted with DCA pellets exhibited a progressive rise in threshold. This amounted

to 14 per cent of the initial value in the first 2 weeks and an additional 5 per cent in the following 17 days. The threshold at the end of 31 days was thus nearly 20 per cent above the initial level and that of the control rats.

TABLE 1. EFFECTS OF INTRAPERITONEALLY INJECTED SALT SOLUTIONS ON ELECTROSHOCK SEIZURE THRESHOLDS (EST) OF NORMAL RATS

SALT	NO. OF RATS	DOSE, mm/kg.	MEAN EST IN MA.		CHANGE IN EST MA	P
			Control	60 min. after injection		
Ringer's solution	16	6	24.5	24.8	$\pm 0.3 \pm 0.2$	>0.1
NaCl	16	6	24.5	27.9	$\pm 3.4 \pm 0.3$	<0.001
CaCl ₂	26	1	25.0	26.0	$\pm 1.0 \pm 0.2$	<0.001
KCl	24	6	27.7	23.5	-4.2 ± 0.3	<0.001
MgCl ₂	22	4	26.7	22.9	-3.9 ± 0.1	<0.001
Na ₂ HPO ₄ —NaH ₂ PO ₄ pH7.4	5	2	25.1	22.9	-2.2 ± 0.2	<0.001
Na ₂ HPO ₄ —NaH ₂ PO ₄ pH7.4	11	4	25.6	23.1	-2.5 ± 0.2	<0.001

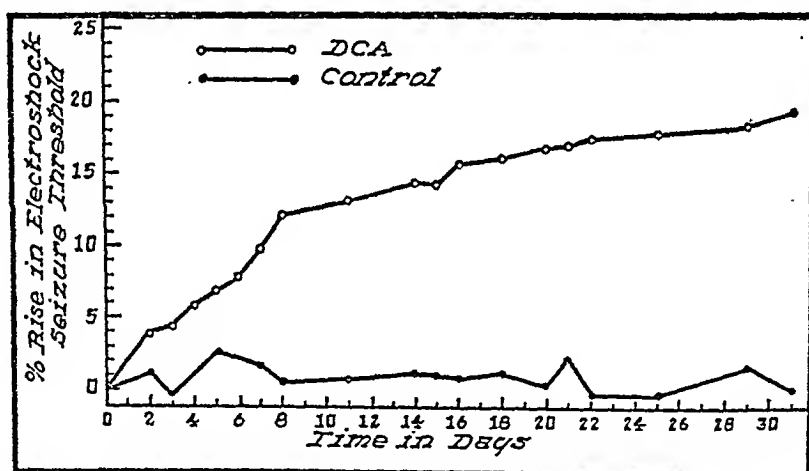


Fig. 2. EFFECTS OF DCA on the electroshock seizure thresholds of rats.

TABLE 2. WATER AND ELECTROLYTE CONCENTRATIONS IN BRAIN CORTEX OF NORMAL AND DCA-TREATED RATS

	N	PER KILOGRAM WET BRAIN			
		H ₂ O	Na.	K	Cl
		gm	mEq.	mEq.	mEq.
Normal rats	10	794 ± 1	50.0 ± 0.2	101.6 ± 0.7	34.2 ± 0.1
DCA, 23-31 days	6	793 ± 2 (0.7)	49.1 ± 0.6 (0.4)	102.2 ± 0.4 (0.6)	33.8 ± 0.2 (0.1)

N = number of pooled samples. Each pool consisted of the brain cortex from 4 rats. Figures in parentheses are P values of differences from controls.

Table 2 shows the observed concentrations of water and electrolytes in the brain cortices of normal and DCA-treated rats. At a time when the electroshock seizure thresholds of the treated animals were very significantly elevated (23-31

days after the implantation of pellets) the concentrations of water, Na, K and Cl in the brains of these animals were the same as those of normal rat brains. Single samples, each representing the pooled brains of 4 rats injected with NaCl, KCl, CaCl_2 or MgCl_2 solutions, were also analyzed and were found to contain normal amounts of Na, K, Cl and water.

Blood was collected from the rats showing the electroshock seizure threshold changes of table 1 or from strictly comparable animals. The results of the analyses of the plasma samples are shown in table 3. Only in the case of CaCl_2 -injected rats was there a significant change in plasma water content. In the DCA-treated animals, the plasma Na was elevated while the K and Mg were depressed to a significant extent. The intraperitoneal injection of NaCl solution caused a rise in plasma Na and a small but probably significant rise in Ca, with no change in plasma K or Mg concentrations. The plasma Ca rose after the injection of CaCl_2 solution but there was no change in the concentrations of the other three cations. After the administration of KCl solution there was a fall in plasma Na, a rise in plasma K, and, surprisingly, a rise in plasma Ca concentration. The injection of MgCl_2 solution resulted in a rise in the plasma Mg concentration accompanied by a fall in both Na and K concentrations.

DISCUSSION

With respect to their effects on maximal seizure patterns, the 4 salts, NaCl, KCl, CaCl_2 and MgCl_2 , fall into 2 groups. The chlorides of the 2 monovalent cations, which abolish the tonic phase of the convulsion, appear to share some of the properties of anticonvulsant drugs (11). The chlorides of the 2 bivalent cations, however, abolish only the tonic extensor component and do not affect the total duration of the convulsion. Since NaCl and CaCl_2 raise the electroshock seizure threshold while KCl and MgCl_2 lower it, it is apparent that the effect of an intraperitoneally injected salt solution on the maximal seizure pattern is not correlated with its effect on the electroshock seizure threshold.

Earlier work done in this laboratory showed that the rat brain has a remarkable ability to preserve a normal content of Na and K under conditions of low plasma Na and markedly elevated plasma K (6). The work presented here indicates that the brain can likewise maintain normal concentrations of Na and K during treatment with DCA, when plasma Na is chronically elevated and plasma K decreased. This observation is in agreement with that of Hoagland and Stone (12) who, using similar analytical methods, found no change in brain Na and K following treatment of rats with DCA. A discussion of the implications of this constancy of brain Na and K concentrations is beyond the scope of this paper.

Clearly, the decreased brain excitability of DCA-treated rats, indicated by the rise in electroshock seizure threshold, is not the result of changes in brain water, Cl, Na or K concentrations. The same is undoubtedly true of the alterations in brain excitability produced by intraperitoneal injection of the 4 salt solutions. This is indicated by the fact that analyses of single samples of brain from the injected rats revealed no differences from normal. The explanation of the changes in brain excitability must lie elsewhere.

TABLE 3. WATER AND CATION CONCENTRATIONS PER KILOGRAM WET PLASMA IN RATS TREATED WITH SALT SOLUTIONS OR DCA

TREATMENT	H ₂ O		Na		K		Ca		Mg	
	gm.	N	mEq.	N	mEq.	N	mEq.	N	mEq.	N
No treatment	921±2	13	142.5±0.5	23	4.70±0.07	23	5.50±0.09	13	2.34±0.04	10
DCA, 23-31 days	922±1 (0.7)	14	152.7±0.9 (0.001)	14	3.35±0.23 (0.001)	14	5.52±0.05 (0.6)	8	1.78±0.07 (0.001)	8
NaCl, 1 hr.	924±1 (0.2)	10	146.5±1.0 (0.001)	10	4.52±0.17 (0.7)	10	5.85±0.06 (0.02)	8	2.30±0.04 (0.09)	8
CaCl ₂ , 1 hr.	931±1 (0.001)	10	141.8±0.5 (0.3)	10	4.36±0.16 (0.1)	10	7.53±0.11 (0.001)	12	2.33±0.03 (0.9)	9
KCl, 1 hr.	920±2 (0.5)	10	135.4±0.8 (0.001)	10	8.64±0.09 (0.001)	10	6.22±0.13 (0.001)	8	2.45±0.05 (0.1)	8
MgCl ₂ , 1 hr.	923±1 (0.7)	10	132.2±0.9 (0.001)	10	3.31±0.08 (0.001)	10	5.52±0.64 (0.9)	9	12.12±0.19 (0.001)	9

N = number of samples; 42% of the samples consisted of the plasma from individual rats, the remainder were the pooled plasmas of 2 to 6 rats. Figures in parentheses are P values of differences from controls.

It has been shown that changes in the degree of hydration produce corresponding changes in the electroshock seizure threshold of the rat (3). However, the changes in electroshock seizure threshold resulting from DCA treatment or the injection of salt solutions cannot be explained on this basis. Only the injection of CaCl_2 solution, which raised the threshold, caused a significant difference in plasma water content, and in this case the increased water content would tend to lower, rather than raise, the electroshock seizure threshold. It has already been shown (6) that no correlation can be made between plasma K concentration and electroshock seizure threshold. This is further emphasized by the fact that the plasma K was significantly lowered in both DCA-treated and MgCl_2 -treated rats. Since in the experiments reported here alterations in plasma Mg concentration were always accompanied by inverse changes in plasma Na, it is impossible to determine whether the plasma Mg concentration exerts any influence on brain excitability. Changes in plasma Ca concentration, if not masked by changes in plasma Na, may affect brain excitability. Of the factors tested, only the increased plasma Ca could account for the elevated electroshock seizure threshold of the CaCl_2 -injected rats. It is also probable that the reduction in electroshock seizure threshold produced by the injection of phosphate buffer was due to a lowering of plasma Ca concentration. Although changes in electroshock seizure threshold can be produced without alterations in plasma Na, it is apparent that in all situations examined here and in adrenalectomized rats (6) any variation of more than 3 mEq/l in plasma Na concentration is accompanied by a change in electroshock threshold. The plasma Na concentration thus appears to be an important factor in determining brain excitability.

SUMMARY

Normal rats treated with DCA or injected with isotonic NaCl or CaCl_2 solution have elevated electroshock seizure thresholds. Rats injected with isotonic KCl or MgCl_2 solution or phosphate buffer show lowered thresholds for electroshock convulsions. The injection of Ringer's solution produces no change in electroshock seizure threshold. Alterations in maximal seizure pattern produced by injection of the salt solutions cannot be correlated with the effects on electroshock seizure threshold. The brains of rats treated for 23 to 31 days with DCA contain normal concentrations of water, Na, K and Cl. This is apparently also true of the brains of rats injected with NaCl, KCl, CaCl_2 or MgCl_2 solutions. The changes in brain excitability produced by treatment with DCA or the 4 salt solutions can be correlated with changes in plasma Na or Ca concentrations. An increase in plasma Ca, if not masked by a change in plasma Na, is accompanied by an elevation of the electroshock threshold. Any change of more than 3 mEq/l in plasma Na is accompanied by a change of electroshock seizure threshold in the same direction.

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EFFECTS OF ADRENALECTOMY AND OF ADRENAL CORTICAL EXTRACT ON DCA-HYPERTENSION IN THE RAT¹

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IT IS a well documented fact that desoxycorticosterone acetate (DCA) is capable of producing an elevation of the blood pressure in a wide variety of species (1). It seems equally certain that the adrenal cortex normally liberates some product or products identical in action with this synthetic steroid (2). In our investigations concerning the action of this compound in the rat we were able to demonstrate not only its potency in the intact animal (3) but also its immediate effect on the kidney (4).

The present study was undertaken to determine whether the adrenal gland might normally liberate some factor opposed to DCA in its action. This idea was first suggested by the observation in the dog (5) and the impression in man (1) that pressor effects seemed more readily elicited by DCA in the absence of normal functioning adrenal cortical tissue. Soffer (1) has stated that this "would suggest that in addition to the hypertensive factor manufactured by the adrenal cortex an additional blood pressure balancing factor is similarly produced," yet little direct investigation of this problem has been undertaken. The earlier work referred to above does not solve the problem for it was only indirectly concerned with the question. Thus, Swingle *et al.* (5) noted that the degree of pressure rise following DCA administration was greater in adrenalectomized than in intact dogs. Since the adrenalectomy was performed some time prior to the DCA administration, however, the blood pressure base line in these animals was lower to start with, although there was no significant difference in the final pressures obtained. A similar criticism applies to the observations in Addisonian patients in whom the duration of the disease represents an uncontrolled variable. The only suitably controlled report seems to be that of Green (6) who was able to demonstrate a minimally increased sensitivity to DCA in adrenalectomized rats only if they were given small doses of the steroid.

Along similar lines, Pines *et al.* (7) have considered the possibility of depressing adrenal cortical function as a possible means of decreasing the blood pressure of hypertensive patients. Continued administration of adrenal cortical extract for one month decreased the 'resting' blood pressure in 3 of 4 hypertensive subjects. Allowing the possibility of a non-specific effect, these authors felt that the result they obtained might represent either "suppression of adrenal cortical function or counteraction of a desoxycorticosterone-like pressor hormone."

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From the above, it seemed of obvious importance to determine whether the adrenalectomized animal was, in fact, more sensitive to DCA before accepting the thesis for an adrenal cortical anti-pressor secretion. Similarly, it appeared of importance to establish by administering cortical extract to animals with DCA hypertension whether the effects observed by Pines *et al.* (7) could have been due to depressor material in the extract.

EXPERIMENTAL

Three separate experiments were performed to determine the relative sensitivity of the adrenalectomized rat to DCA administered in pellet form and a 4th to determine the effect of cortical extract administered to DCA-treated animals.

Experiment 1. Thirty-three male albino rats of the Sherman strain were maintained for a period of 44 days. *Group 1*, consisting of 8 animals, served as untreated controls. *Group 2*, consisting of 14 animals, received a subcutaneous implant of DCA (one third of a 75-mg. Schering Cortate pellet) on the 1st, 16th and 33rd days

TABLE 1

TREATMENT	CONTROL	DCA-SALINE	ADRENALECTOMY + DCA-SALINE
Initial wt., gm.	130	124	125
Final wt., gm.	170	177	195
Blood pressure, mm. Hg			
4th week	96	107	119
	± 14	± 14	± 16 p < 0.01
7th week	105	122	125
	± 9	± 19	± 14
Heart wt., mg/100 cm. ²	150	171	200
	± 13	± 13	± 9
Kidney wt., mg/100 cm. ²	358	476	501
	± 13	± 24	± 23

of the experiment. The 11 animals of *group 3* were adrenalectomized on the first day of the experiment and were subsequently treated in the same way as those of *group 2*. Saline (1%) was substituted for the drinking water of both DCA-treated groups. Blood pressure was determined by the method of Byrom and Wilson (8) using ether as anesthetic, on the 25th, 29th and 31st days and again on the 44th day of the experiment. At the conclusion, hearts and kidneys were weighed after 24 hours' fixation. Table 1 presents the findings. Since the three separate blood pressure determinations in the 4th week yielded the same result they are aggregated as a single datum in the table.

As has previously been reported (9) rats of the Sherman strain are rather resistant to the pressor effects of DCA. Nonetheless, it is quite apparent that in the earlier period (4th week) the adrenalectomized animals are slightly more sensitive to the pressor effect of DCA than are the non-adrenalectomized, in this aggregate datum. This difference disappears in the 7th week. At the end of the experiment, heart and kidney weight was increased in both DCA-saline treated groups, the increase being greater in the adrenalectomized animals.

Experiment 2. In order to obviate the possibility that the substitution of saline for drinking water in the previous experiment had been an additional complication, a second experiment was undertaken in which DCA alone was administered to adrenalectomized and intact animals. Three groups of 8 male albino rats of an inbred Wistar strain were maintained for 26 days. *Group 1* served as untreated controls, *group 2* received DCA implants (one third of a 75-mg. pellet) on the 1st, 5th, 10th, 15th and 20th days and *group 3* received the same treatment as *group 2* but these animals were bilaterally adrenalectomized on the first day of the experiment. Blood pressure was determined at weekly intervals by the method already described. At the conclusion of the experiment the animals were killed and hearts and kidneys weighed after 24 hours' fixation. The data are presented in table 2.

TABLE 2

TREATMENT	CONTROL	DCA	ADRENALECTOMY + DCA
Initial wt., gm.	70	94	98
Final wt., gm.	158	180	185
Blood pressure, mm. Hg			
7 days	95 ±11	125 ±16	103 ±10
13 days	101 ±6	124 ±13	122 ±12
19 days	96 ±9	127 ±20	134 ±21
26 days	89 ±13	117 ±20	134 ±13
Heart wt., mg/100 cm. ²	181 ±9	215 ±20	235 ±11
Kidney wt., mg/100 cm. ²	453 ±51	511 ±73	520 ±50

Although there was a suggestion that the adrenalectomized animals were again more susceptible to the pressor effect of DCA in the 4th week, the difference between the two DCA-treated groups is not significant. Kidney and heart weights were increased to the same degree in both groups. It is apparent that when no saline is administered there is no real difference between the sensitivity of intact and adrenalectomized rats to the pressor effects of DCA. It is, of course, possible that if a larger series of animals were tested the suggested difference between groups might actually become significant.

Experiment 3. The preceding two experiments taken together had shown that any slight increase in the susceptibility of adrenalectomized animals to the pressor effects of DCA was evident only when saline was substituted for the drinking water and then only in an early phase of the treatment.

It had also been noted that, following treatment with DCA and saline, adrenalectomized animals showed a greater increase in kidney weight. A third experiment was now undertaken in Wistar animals to study this renal effect and to confirm the

previous findings with regard to blood pressure. Treatment was carried out for 38 days in three groups of 10 animals. *Group 1* consisted of intact controls, *group 2* of DCA-treated intact animals and *group 3* of DCA-treated adrenalectomized rats. One per cent saline was substituted for the drinking water of the DCA-treated animals and a record was kept of the water intake of the three experimental groups. DCA pellets in this experiment were implanted on the 1st, 14th and 28th days.

TABLE 3

TREATMENT	CONTROL	DCA-SALINE	ADRENALECTOMY + DCA-SALINE
Initial wt., gm.	61	72	64
Final wt., gm.	204	178	174
Blood pressure, mm. Hg			
35 days	100 ±12	157 ±33	126 ±18
37 days	102 ±10	149 ±27	130 ±17
Heart wt., mg/100cm. ²	186 ±27	243 ±35	248 ±32
Kidney wt., mg/100cm. ²	492 ±78	648 ±91	710 ±84
C_{IN} cc/min.	0.38 ±0.07	0.37 ±0.08	0.38 ±0.06
C_{PAH} cc/min	3.04 ±0.46	3.30 ±0.63	3.12 ±0.68
Tm_{PAH} mgm/min.	0.137 ±0.036	0.170 ±0.020	0.154 ±0.034
C_{IN} cc/min.	0.81	0.59	0.55
C_{PAH} cc/min.	6.45	5.37	4.10
Tm_{PAH} mg/min.	0.315	0.275	0.223
FF as %	13.04 ±3.2	11.1 ±2.0	13.03 ±3.9
C_{PAH}/Tm_{PAH}	21.2 ±4.2	20.1 ±1.9	20.0 ±1.9
Water intake cc/day/100gm.			
1st week	28	39	35.5
2nd week	24	34	39
3rd week	23	36	35
4th week	19	35	30.5
5th week	17	41	28.5

Renal function was determined at the conclusion of the experiment according to methods previously described (10). Following this procedure, the animals were killed and the kidneys and hearts weighed after 24 hours' fixation. Table 3 presents the data.

It is again apparent that adrenalectomized animals are not more susceptible to the pressor effects of DCA; indeed, blood pressure was significantly lower than in the intact DCA-treated group. Although renal function remained normal in both DCA-treated groups, renal hypertrophy did occur, so that renal function per gram of

kidney tissue was considerably reduced. Renal hypertrophy was greater in the adrenalectomized than in the intact animals, despite the fact that in the latter part of the experiment their saline intake declined considerably.

Experiment 4. Thirty-six male albino rats of an inbred Wistar strain were divided into three equal groups and maintained for 28 days. *Group 1* served as untreated control while *group 2* received DCA by the subcutaneous implantation of a pellet (one third of a 75-mg. pellet) on the 1st, 6th, 11th, 16th and 21st days of the experiment. The animals of *group 3* received DCA in the same manner as those of *group 2* but for the duration of the experiment were given daily injections of cortical

TABLE 4

TREATMENT	CONTROL	DCA	LIPO-CORTI- CAL EXTRACT + DCA
Initial wt., gm.	52	49	53
Final wt., gm.	175	153	159
Blood pressure, mm. Hg			
14 days	104 ±12	117 ±10	124 ±12
23 days	101 ±14	122 ±19	128 ±13
27 days	96 ±17	133 ±14	127 ±14
Heart wt., mg/100cm ²	180 ±19	203 ±14	205 ±17
Kidney wt., mg/100cm ²	459 ±65	534 ±25	535 ±45
C _{IN} cc/100cm ² /min.	0.32 ±0.06	0.28 ±0.05	0.31 ±0.07
C _{PAH} cc/100cm ² /min.	2.82 ±0.36	2.91 ±0.26	2.83 ±0.40
Tm _{PAH} mgm/100cm ² /min.	0.173 ±0.022	0.174 ±0.024	0.161 ±0.017
FF as %	11.4 ±1.9	9.54 ±1.9	11.0 ±1.8
C _{PAH} /Tm _{PAH}	16.3 ±0.5	16.7 ±1.0	17.6 ±2.1

extract. For this purpose, $\frac{1}{10}$ cc. of Upjohn Lipo-Cortical extract was injected subcutaneously once daily for 5 days each week. Each cc. of this extract contains 40 rat units by biological standardization, equivalent to 2 mg. of 11 dehydro 17 hydroxycorticosterone (Kendall's compound E). The choice of a daily administration of $\frac{1}{10}$ cc. (4 RU or 0.2 mg. Kendall's E) was determined by the amount of material available for this work. At the end of the third week, when it became apparent that the extract had not modified the hypertensive effect of the DCA, the dose of extract was doubled for the 4th and last week of the experiment.

Blood pressure was determined on the 14th, 23rd and 27th days of the experiment. On the 28th day renal function was estimated. Table 4 presents the pertinent data.

Aside from confirming findings which have previously been reported, such as the effectiveness of DCA in producing hypertension even without any intensifying measures, and the absence of renal functional change during the earlier stages (4 weeks) of such treatment, this experiment is distinguished by its complete negativity. The lipocortical extract, in the amounts given, in no way modified either the blood pressure or renal functional status of animals treated with DCA for 4 weeks. These observations are confirmed by both kidney and heart weight which are reasonably sensitive indicators of interference with renal function on the one hand or elevation of blood pressure on the other. The experiment again demonstrates that the maintenance of normal renal function in DCA-treated animals requires an increase in kidney mass.

DISCUSSION

Adrenalectomy. These experiments have demonstrated the difficulty of establishing the relative sensitivity of adrenalectomized animals to the pressor effect of DCA. A slightly increased susceptibility was observed in the early phase of treatment but this appeared as a significant finding only when saline was administered with the DCA. No increase in sensitivity was elicited in the later phases of treatment (6th and 7th weeks) despite the administration of saline as drinking water. A greater degree of renal hypertrophy was observed, however, in adrenalectomized animals given DCA with saline for this longer period.

These findings are in agreement with those of Green (6) who was able to show only a slight increase in the blood pressure response of the adrenalectomized rat given DCA with saline, but not with the indirect observation in the dog (5) and man (1). It seems reasonable to suggest that the minimal early sensitivity observed only when salt is given with the DCA hardly indicates a specific anti-pressor activity for the adrenal cortex. Rather it would seem that the effects observed can more readily be ascribed to the general metabolic disturbance following adrenalectomy.

While these findings argue against the liberation of specific depressor substances from the adrenal cortex they are in no way conclusive. Exogenously administered DCA might easily suppress such cortical depressor functions in the intact animal to begin with, so that by the criteria used here no difference could be expected between intact and adrenalectomized animals. Similarly, a homeostatic mechanism satisfactory for normal needs might be overshadowed, if not actually depressed, by the quantities of pressor material here added to the organism as exogenous DCA. Nonetheless, the experiments do indicate that if materials capable of counteracting DCA do exist in the organism they must be of a more subtle nature than has previously been indicated in the literature.

Cortical extract. Under these circumstances, it is not surprising that the gross procedure of injecting adrenal cortical extract to DCA-treated animals was entirely without effect. If materials capable of counteracting the DCA hypertensive effect are present in such an extract, their subtle presence is masked, not only by those corticoids with no effect on the blood pressure, but by the liberal content in such extracts of substances with DCA-like action. It seems apparent, then, that the

depressor action observed by Pines *et al.* (7) in hypertensive patients could hardly have been due to a direct action of the material itself. This conclusion would underline the alternate explanation of these authors that the extract suppressed adrenal function.

SUMMARY

Experiments in intact and adrenalectomized rats revealed only a minimal increase in the sensitivity of adrenalectomized animals to the pressor effect of DCA. This was observed as a significant finding only when sodium chloride was added to the drinking water, and only as a temporary phase during the course of treatment. A greater degree of renal hypertrophy occurred following such treatment in the adrenalectomized rat, but again only in the presence of added saline. These results do not support the concept of a specific antipressor activity in the normal adrenal gland, but rather reflect the precarious water balance of the adrenalectomized animal. The administration of adrenal cortical extract to DCA-treated animals did not modify the effects of this latter substance, indicating the absence of any grossly detectable depressor substance in the extract used.

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SPREAD OF EXCITATION IN THE DOG HEART¹

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SEVERAL methods have been employed in the past to determine the time of arrival of the excitation wave at various points on the surface of the dog heart (1-4). There is general agreement that the earliest point on the ventricular surface to become electronegative is the mid-portion of the anterior right ventricle near the septum. There is further agreement that most of the right ventricular surface becomes depolarized from 8 to 10 milliseconds earlier than the left ventricular surface. Most of the methods employed, however, were carried out on animals with exposed hearts, a technique which introduces at least two complications in the proper interpretation of the results: 1) in the exposed heart normal conducting tissues are removed from the surface of the heart, and 2) most of the experiments were done under the cooling effect of room air. In view of the fact that lead II is used as a standard of reference for the measurement of the time of arrival of the excitation wave at the various points on the heart surface, an alteration in lead II caused by these two factors might introduce errors in the measurements because the earliest deflection in the ventricular complex in lead II may not represent the onset of ventricular excitation. Furthermore, if the surface of the heart is cooled by exposure to room air, conduction from the endocardium to the epicardium may be slowed in the exposed area so that the results thus obtained may not correspond to the true order of excitation in the intact animal. In spite of these objections, much useful information has been afforded by such experimentation. However, the methods do not readily lend themselves for study of the effect of such agents as anoxia and drugs on the spread of excitation in the intact animal.

A method for the study of the spread of excitation in the intact animal became available as a result of studies on the nature of unipolar extremity leads in the dog (5-7). In these studies it was found that from an analysis of the QRS complex in any unipolar lead one could ascertain what parts of the heart were being depolarized from moment to moment; this information was afforded by the discovery that preponderant depolarization in the proximal zone of any unipolar lead resulted in downward movement of the beam, while upward movement was caused by preponderant depolarization in the distal zone. When the excitation involved the intermediate zone of the lead or was of equal degree in both proximal and distal zones, the beam remained at the iso-electric line. Since the distribution of the proximal, intermediate, and distal zones for each of the unipolar extremity leads was also established, localization of the

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excitation process to various parts of the heart became possible. When analyzing a single unipolar lead, however, the excitation process can be localized only to rather large segments of the heart. When the three unipolar leads, VR, VL, and VF, are recorded simultaneously and an analysis is made of the moment-to-moment beam position in each of the leads, then the excitation process can be localized to much smaller segments of the heart. Figure 1 shows that because of the overlapping zones of the three unipolar extremity leads, the various segments of the heart bear a definite relation to each of the three leads. Depolarization in each of these segments results in a specific combination of beam movements in the three leads. For example,

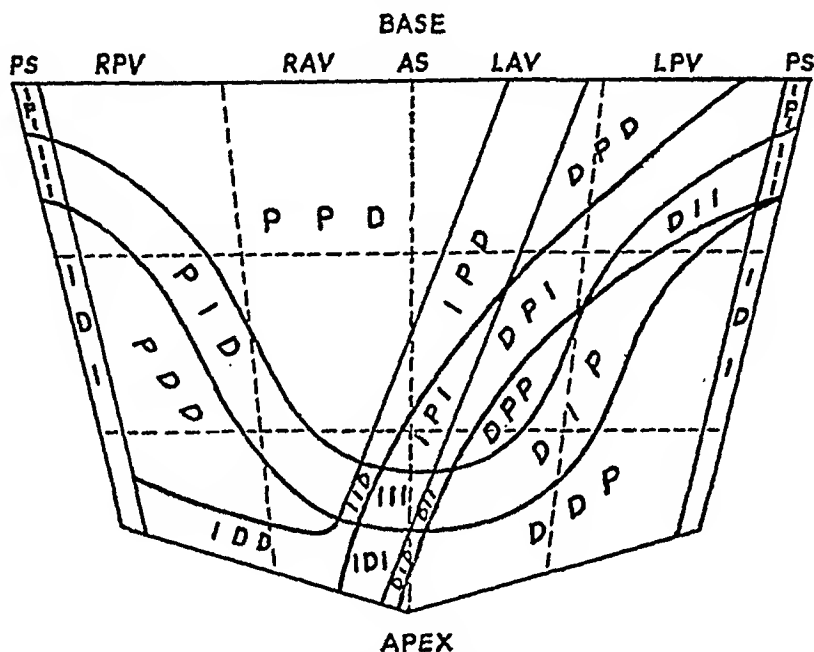


Fig. 1. SCHEMATIC DRAWING OF VENTRICULAR SURFACE OF DOG HEART showing overlapping of the proximal, intermediate and distal zones of leads VR, VL, and VF (composite picture of findings of 40 dogs). PS, posterior septum; RPV, right posterior ventricle; RAV, right anterior ventricle; AS, anterior septum; LAV, left anterior ventricle; LPV, left posterior ventricle; P, proximal zone; I intermediate zone; D, distal zone. The various areas diagrammed are labelled according to their zonal representation in leads VR, VL, and VF. The first of the three letters which label each of the areas represents the zone of lead VR, the second letter the zone of lead VL, and the third letter the zone of lead VF (e.g., area labelled PPD indicates that this portion of the heart lies in the proximal zones of leads VR and VL, but in the distal zone of lead VF).

if the beam position in each of the three leads at a given moment indicates preponderance of depolarization in the proximal zone of lead VR and lead VL (downward movement of the beam in VR and VL) and in the distal zone of lead VF (upward movement of the beam in lead VF) the site of preponderant depolarization is localized to that segment of the heart labelled PPD in figure 1 (the upper anterior right ventricle). If at a given moment in the QRS complex the beam has moved upward in VR and VL (indicating preponderance of depolarization in the distal zones of VR and VL) and at the same time has moved downward in lead VF (indicating preponderance of depolarization in the proximal zone of VF), the site of preponderant depolarization is thus localized to segment DDP in figure 1 (the left apical region). In this manner every combination of beam movements found in the three leads will help to localize

the excitation process to one or another of the various heart segments depicted in figure 1.

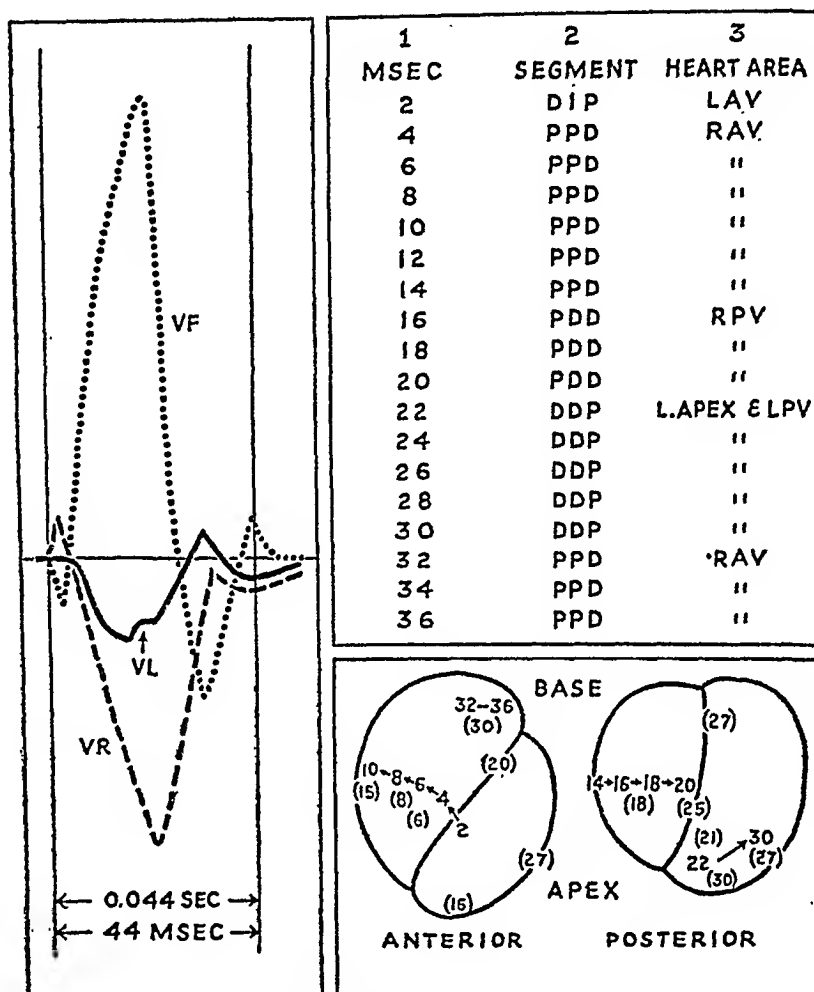


Fig. 2. ANALYTICAL METHOD OF DETERMINING SPREAD OF EXCITATION IN VENTRICLE. VR, VL, and VF are recorded simultaneously and superimposed. The position of the beam in the 3 leads is determined from moment to moment throughout the ventricular complex. MSEC—time in milliseconds during QRS interval; SEGMENT—the three letters *P*, *I*, *D* mean proximal, intermediate, and distal. For each time interval the first of the three letters represents the zone of lead VR in which the excitation is present; the second letter the zone of lead VL, and the third letter the zone of lead VF. Thus as 2 milliseconds segment DIP means that excitation is present in the distal zone of VR, the intermediate zone of VL, and the proximal zone of VF (the lower anterior left ventricle). HEART AREA—area of heart corresponding to segment in column two. LAV, left anterior ventricle; RAV, right anterior ventricle; RPV, right posterior ventricle; LPV, left posterior ventricle. In the lower right-hand corner of the diagram the anterior and posterior surfaces of the dog ventricle are represented. The bold figures are in milliseconds and represent the time of arrival of the excitation process in the various segments of the heart as determined by the analytical method. The figures in parentheses represent the time of arrival of the excitation wave at various points of the surface of the heart in the same animal (with chest closed) as determined by the direct method of Lewis and Rothschild. Note close correspondence between values obtained by the two techniques.

By this type of analysis one can detect the site of preponderant depolarization from moment to moment during ventricular excitation. The sequence of this spread of excitation probably corresponds closely to the actual spread of the excitation wave

through the ventricles for the following reasons: *a*) the initial beam movement certainly can be considered to represent the first site activated, *b*) the speed of conduction through both the right and left bundles is probably the same (since it is known that the fibers in each bundle are of the same size). Therefore preponderance of depolarization in any segment must mean that the excitation wave has reached this area in advance of other areas, for otherwise there would not be enough depolarized fibers to produce the specific movement of the beam, and *c*) as will be seen below, the order of preponderant depolarization corresponds closely to the actual arrival of the excitation process at the surface of these segments.

METHOD

The ventricular complexes in the three unipolar extremity leads VR, VL, and VF are recorded simultaneously on a Sanborn Tribeam at rapid speeds (75 mm/sec.) in order to make the measurements technically easier. These complexes are then photographically enlarged and transposed to graph paper. This makes it possible to subdivide the QRS complex into intervals of 4 milliseconds and then to record the position of the beam (as going up or going down) in each of the three leads at successive 4-millisecond intervals. The beam position in each lead is labelled by the letters *P*, *D*, or *I*, depending upon the direction of the beam deflection at each moment. If the beam is being deflected downwards, the letter *P* is used to signify preponderance of depolarization at that moment in the proximal zone of the lead. If the beam is being deflected upwards in a lead, the letter *D* is used to signify preponderance of depolarization in the distal zone of the lead. If the beam remains at the iso-electric line at the onset of ventricular excitation, the letter *I* is used to indicate depolarization in the intermediate zone of the lead. The resulting combination of three letters identifies that segment of the heart which is the site of preponderant depolarization at each successive time interval. The time in milliseconds needed for the excitation wave to involve the various segments of the heart is charted on a diagram of the anterior and posterior surface of the heart (fig. 2).

RESULTS

When this technique was applied to the electrocardiographic complexes obtained in a series of 30 dogs it was found that the usual order of segment depolarization was as follows:

TIME IN MSEC. (AFTER ONSET OF VENTRICULAR EXCITATION)	SEGMENT (IN FIG. 1)	HEART AREA
3	DIP, IPD, or PPD	anterior septum or anterior right ventricle near septum
6-12	PPD	upper anterior right ventricle
15	PDD	posterior right ventricle
18-27	DDP	left apex and posterior left ventricle
30-33	PPD	upper anterior right ventricle (pulmonary conus)

It was observed that beyond 33 to 35 milliseconds occasionally the beam remains above the iso-electric line in all three unipolar extremity leads. Since no segment of the heart has been found to lie in the distal zone of all the leads, localization of the excitation process at such points in the QRS complex is not possible. During this period there may be repolarization of some parts of the heart while depolarization is not yet complete in other parts, resulting in potentials of mixed origin so that the particular beam position can no longer be used in localizing the segment being de-

polarized. It is also possible that the potentials of depolarization at this time at each electrode, though present, are of such low magnitude that they approximate the potential of the central terminal so that the resulting beam positions might be due to the potential variations of the central terminal rather than to the variations of the exploring electrode.

The first region depolarized usually is the mid-anterior septum or the anterior right ventricle. This is followed in turn by the posterior right ventricle, and then by the left apex and posterior left ventricle. In many hearts terminal excitation again involves segment PPD (fig. 2). Since most of this segment was depolarized early, the terminal excitation probably involves the pulmonary conus which is part of the segment.

In order to test the validity of this analytical technique of determining the spread of excitation in the intact animal, the time of arrival of the excitation wave at various points on the heart surface was then determined in four of the same animals by the direct method of Lewis and Rothschild (1). The results obtained by this method were compared with the results obtained on the same animals before their chests were opened by the analytical method described above. In utilizing the method of Lewis and Rothschild, tiny pin electrodes were inserted through the pericardium at various points in such a manner that the electrode was in contact with the epicardium without injuring it. The indifferent electrode consisted of a central terminal electrode constructed as described previously (5). The lungs were restored to their normal position, the chest closed tightly, and the animal allowed to breathe on his own. During the exposure of the heart, a lamp was allowed to shine over the operative field to prevent undue cooling of the anterior heart surface, and records were taken only after a period of an hour or so had elapsed after closure of the chest. It had been found in previous experiments (8) dealing with heart temperature that the normal temperature of the anterior surface of the heart is not restored until at least one hour after closure of the chest wall. These precautions were taken to minimize any possible effects of lowered heart temperature upon the spread of conduction and depolarization (9). Comparison of the results obtained by the two techniques on the same animal reveal close correlation (fig. 2).

DISCUSSION

The spread of excitation in the dog heart as determined by the analytical method described above is found to correspond fairly closely with the spread of excitation as determined by Lewis and Rothschild and others. It can be seen that excitation first involves the anterior septal region and extends rapidly to depolarize the anterior and posterior right ventricle before most of the left apex and posterior left ventricle become depolarized. It should be noted that points on the surface of the left lateral ventricle receive the excitation wave later than do corresponding points on the right lateral ventricle. If one assumes that the subjacent endocardial points in the right and left ventricles are probably at the same distance from the A-V node, and that the speed of conduction along both bundles is the same—both reasonable assumptions—then the later arrival of the excitation wave at the surface of the left ventricle could be attributed to the greater thickness of the left ventricle. Lewis and Rothschild considered that by their technique they were measuring the time of arrival of the

excitation wave at the various points on the surface of the ventricles and that the later arrival at the surface of the left ventricle was indeed due to the greater thickness of this ventricle (1). Using the analytical method, the time of arrival of the excitation wave at various segments of the heart, rather than at specific surface points, has been determined. Nevertheless, the results of the two methods yield comparable values, suggesting that what is being measured by the analytical method is also depolarization at the epicardial surface of the various segments. If this be so, one can conclude that surface depolarization dominates the formation of the electrocardiogram in leads recorded from the surface of the body, a view already expressed by several authors (10, 11).

The ventricular complexes in the three unipolar extremity leads of most dogs tend to resemble each other very closely. However, there are occasionally animals that exhibit somewhat different complexes. Since these changes in the form of the ventricular complexes can be due to slight alterations in the position of the heart in these animals as compared to the average animal, one is not justified in concluding that the difference in the complexes indicates a different sequence of excitation, although this possibility cannot be excluded. These variations in the complexes of different animals do not, however, invalidate the use of the method in studying the effect of various agents upon the spread of excitation in the intact animal since each animal serves as his own control.

SUMMARY

The spread of excitation in the dog ventricle has been determined in the intact animal by an analysis of the ventricular complexes simultaneously recorded in the unipolar extremity leads VR, VL, and VF. The method depends upon the fact that depolarization in different parts of the heart produces specific combinations of simultaneous beam movement in the three unipolar extremity leads. An analysis of the beam positions from moment to moment in the QRS complex in each lead permits the recognition of the time when depolarization occurs in the various segments of the ventricles, and, therefore, the time of arrival of the excitation wave at the various segments. The results obtained by this method correspond closely to those obtained in the same animals when the direct method of Lewis and Rothschild is employed.

The order of excitation is usually as follows: mid-anterior septum, anterior right ventricle, posterior right ventricle, left apex and posterior left ventricle, and pulmonary conus.

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EXTRACELLULAR WATER CONTENT OF THE HEART IN DOGS SUBJECTED TO HEMORRHAGIC SHOCK MEASURED WITH THE RADIOACTIVE ISOTOPE OF SODIUM^{1,2}

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WIGGERS suspected the existence of a cardiac factor in the irreversible shock produced by his technique of hemorrhagic hypotension because he observed a progressive fall in cardiac output at a time when venous return and arterial pressure remained adequate (1).

Altered capillary permeability is a prominent feature of myocardial injury in a number of circumstances. The human heart is edematous in failure (2). Hearts which have failed in isolated heart preparations also exhibit edema (3). Regions of the ventricle made temporarily ischemic by experimental occlusion of a coronary artery have been found to contain an excess of extracellular water after as short a period of occlusion as five minutes (4). Asphyxia and anoxia produce profound alterations in the myocardial capillaries (5, 6). It therefore seemed possible that myocardial capillary injury might occur during the severe hypotension to which dogs are subjected in the Wiggers procedure. If this were the case, the myocardium might become edematous and the edema itself cause progressive failure of the heart for purely physical reasons.

The technique introduced by Manery and Bale (7) for measurement of the so-called 'sodium space' makes investigation of the volume of the extracellular fluid relatively simple. We therefore applied this technique to determine whether myocardial edema does develop in dogs subjected to the Wiggers procedure.

METHODS

The dogs used in this study were unselected mongrels and were for the most part well nourished and well hydrated. Anesthesia was obtained by using 125 to 150 milligrams of sodium barbital and 3 milligrams of morphine sulfate per kilogram intravenously. No experiment was begun less than one hour following the injection, and fairly uniform anesthesia was obtained. Heparin was used as an anticoagulant, and we were unable to see that it was a significant factor in the production of any pathological change.

In the production of 'hemorrhagic shock' in dogs, we successfully reproduced the procedure described by Wiggers *et al.* (8). Fifteen dogs were used to develop facil-

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ity with this technique (*Series A*). A similar series of 9 dogs (*Series B*) received radioactive sodium. The isotope was supplied as sodium carbonate. Prior to administration it was converted to isotonic sodium chloride and the pH adjusted to 7.4 by the addition of hydrochloric acid. After this the solution was carefully administered intravenously. At least one hour was allowed for the isotope to equilibrate in the sodium space.

When the animal died or was killed, whole blood was drawn under mineral oil, and three samples of ventricular muscle were excised from the heart, which was often still beating. All possible blood was blotted from the sections, using gauze, and the chordae tendineae, fat and valves were removed from the tissue. One portion of the tissue was dried to constant weight in order to determine the 'total water' of the myocardium.

To calculate the extracellular and intracellular water according to the method of Manery and Bale (7), it was necessary to determine the relative concentration of radioactive sodium in the heart muscle and in the serum.

Samples of heart muscle were prepared for counting by two different methods. The first procedure involved extraction of the radioactive sodium. A sample of heart muscle was weighed, water added to bring the total weight to 200 grams, and the mixture macerated in a Waring Blendor for 20 minutes. The resulting mixture was centrifuged at 3500 rpm for 30 minutes. One milliliter samples of the supernatant fluid were placed in cups and dried slowly for 45 minutes in an oven. Then their activities were determined on a Geiger-Mueller Counter.

In the second procedure, duplicate samples of heart muscle were prepared for counting by dry ashing the tissue overnight in a muffle furnace at 500 to 600° C. The activity of the ash was then determined. Satisfactory checks were obtained on the activity of duplicate ashed samples. The activity of the ashed heart muscle was in all cases slightly higher (3-10%) than the activity of water-extracted heart muscle. Dry ashing was then adopted as the method of choice because of its efficiency and convenience. As a further check on 'background counts' samples of heart muscle were also taken from normal untreated dogs, but no natural radioactivity could be detected in these samples.

The whole blood obtained was centrifuged, and 1 ml. samples of serum placed in cups, dried and counted. This procedure gave uniform samples for counting, and the activity of duplicate samples checked closely.

Each sample container was counted several times and these counts were averaged as the count of the sample. This was compared with a duplicate sample treated in the same manner. The standard deviation of the difference between these two estimates was determined and corrected for sample size. In the case of the heart muscle extracts, in 95 per cent of the cases the difference between duplicates was less than 11 counts per minute and the error of the mean of two such duplicates was 8 counts per minute. Over the range of activities observed in these extracts, this represents an error of about 8 per cent. In the case of the ashed heart samples the corresponding figure was about 5 per cent and for serum samples it was even less.

The extracellular water of the ventricular myocardium was calculated according to the method of Manery and Bale (7), but no correction was applied for the Gibbs-

Donnan equilibrium or for the volume occupied by the protein of the serum. We felt that no such correction was justified because the effects tended to cancel each other in the case of sodium. Furthermore, Kaltreider and Meneely (9) were unable to find systematic differences between the activity of serum and the activity of transudates. We have followed the symbols used by Manery and Bale (7); $(\text{H}_2\text{O})_E$, designates extracellular water; $(\text{H}_2\text{O})_I$, intracellular water, and $(\text{H}_2\text{O})_T$, total water. All these values were expressed as percentage of the wet weight of the ventricular muscle.

It was further necessary to know the activity of the erythrocytes, since 107 milliequivalents per liter of sodium are found normally in such cells in the dog. It was possible to compute the activity of the red cells by determining packed cell volume, the activity of whole blood, and the activity of serum. This was done in a series of 6 control and 7 shock dogs.

Eleven dogs (*Series C*) were used as controls. They were anesthetized in the same manner as the shock dogs, were given radioactive sodium intravenously and in an average of 1 hour and 5 minutes were bled from an artery, the beating heart excised and samples of serum and heart muscle treated as under *Series B*.

Six dogs (*Series D*) served as negative controls and were given large amounts of saline intravenously. The animals were anesthetized and given radioactive sodium intravenously. From a standard infusion set at a height of about 3 feet above the level of the auricle normal saline solution was permitted to flow as fast as the apparatus would permit until the animals began to show some ill effects, such as abdominal distension, cyanosis, cardiac irregularity, or dyspnea. The infusion was then halted for intervals ranging from 30 minutes to an hour and then continued in the same manner until the animal's death.

Blood and myocardial samples were obtained and treated as were similar samples under *Series B*. Complete routine autopsies were performed on all dogs. The erythrocytes contributed from 11 to 45 per cent of the activity of whole blood. The activity contributed by the red cells was directly related to the packed cell volume. In all cases there was hemoconcentration in shock dogs, and therefore the activity of whole blood as compared with that of serum was relatively higher in shock dogs than in the control animals. Actual determinations of hemoglobin residual in ventricular muscle samples were made in 6 dogs. These data showed that residual blood in such samples contributed so little activity to the sample that it was less than the error involved in the counting. Therefore, we felt justified in basing the calculation of $(\text{H}_2\text{O})_E$ and $(\text{H}_2\text{O})_I$ on the activity of serum rather than that of whole blood.

RESULTS

The distribution of water in the myocardium in *Series B*, *C*, and *D* is presented in table 1. It is at once evident that there is no increase of myocardial extracellular water in dogs subjected to the Wiggers procedure (8) of hemorrhagic hypotension to induce irreversible shock. The mean extracellular water content of 24.7 ± 4.4 per cent of wet weight of tissue in these shock dogs is not different from the mean of 23.5 ± 4.5 per cent present in controls. The control value is very similar to the data of Manery and Bale (7). The method is adequate for detection of edema as is shown

by the negative control dogs where the mean extracellular water content in the myocardium was 33.5 ± 3.6 per cent. This is significantly different from the positive control series.

DISCUSSION

The concept of developing cardiac edema as an explanation of the deterioration of the heart in irreversible shock was an attractive one. In a preliminary presentation (10) we actually suggested this possibility. Our hypothesis was certainly not borne out by the experiments reported above. It is evident that a subtler cause for myocardial deterioration in irreversible shock must be sought.

TABLE I

Series B DOGS SUBJECTED TO WIGGERS PROCEDURE				Series C POSITIVE CONTROLS (NO HYPOTENSION)				Series D NEGATIVE CONTROLS (SALINE INFUSION)			
No.	(H ₂ O) _E	(H ₂ O) _I	(H ₂ O) _T	No.	(H ₂ O) _E	(H ₂ O) _I	(H ₂ O) _T	No.	(H ₂ O) _E	(H ₂ O) _I	(H ₂ O) _T
1	22.8	57.8	80.6	1	28.1	49.4	77.6	1	31.6	47.7	79.3
2	31.8	46.7	78.5	2	27.3	49.2	76.5	2	33.1	48.2	81.3
3	32.1	46.9	79.0	3	17.3	64.7	82.0	3	40.1	39.1	79.2
4	26.2	49.2	75.4	4	29.4	49.8	79.2	4	34.9	52.4	87.3
5	20.7	54.3	75.0	5	24.8	57.7	82.5	5	30.4	51.3	81.7
6	23.6	49.9	73.5	6	16.5	55.3	71.8	6	31.0	55.8	86.8
7	21.7	56.9	78.6	7	27.5	47.0	74.5				
8	21.4	55.4	76.8	8	25.8	47.6	73.9				
9	22.2	56.7	78.9	9	19.3	55.9	75.1				
				10	23.4	54.6	78.0				
				11	24.2	55.9	80.1				
	24.7	52.6	77.4		23.5	53.8	77.3		33.5	49.1	82.5
	± 4.4	± 5.5	± 4.0		± 4.5	± 5.5	± 3.6		± 3.6	± 5.6	± 5.4

Series B dogs were subjected to hemorrhagic hypotension according to Wiggers' (8) method.

Series C dogs were treated in a manner identical with *Series B* dogs except that they were not subjected to hypotension. *Series D* dogs were given massive saline infusions to induce edema.

(H₂O)_E is extracellular water, (H₂O)_I is intracellular water, (H₂O)_T is total tissue water: all expressed as % wet wt. of ventricular muscle.

SUMMARY

The method of Manery and Bale (7) was employed to measure the extracellular water of the myocardium in 3 groups of dogs treated in different ways: The first group was subjected to severe hemorrhagic hypotension to produce irreversible shock by the technique of Wiggers (8). The second group represented positive controls in that they were treated in the same manner as the shock dogs but were not bled. The third group, which served as negative controls, were given large and rapidly administered intravenous infusions of saline.

The positive control dogs showed an extracellular water content in the myocardium of 23.5 per cent of the wet weight of tissue. This is in quite close accord with the data of Manery and Bale (7). The negative control dogs developed an edema of myocar-

dium obvious on gross and microscopic examination. The extracellular water in these hearts was 33.5 per cent of the wet weight of the tissue. This is significantly and convincingly different from the positive controls. Thus the method is adequate to detect edema when it is present.

The extracellular water of the myocardium of the dogs subjected to Wiggers' (8) hemorrhagic hypotension procedure was 24.7 per cent of the wet weight of tissue. There is, therefore, no edema of the heart in dogs subjected to this form of irreversible shock.

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VASODEPRESSOR RESPONSES TO MORPHINE FOLLOWING HEMORRHAGIC HYPOTENSION^{1, 2}

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MORPHINE sulfate is used extensively in cases of post-traumatic and hemorrhagic hypotension to relieve the pain and emotional tension which many patients exhibit. Considerable differences have been encountered in the ability of these patients to tolerate the drug. In many instances, no untoward reactions are discernible; in others the administration of morphine leads to an exacerbation of the symptoms of shock. There would therefore appear to be significant variation in the response to morphine depending on the precise state of shock at the time the morphine is given.

In experimental studies the usual doses of morphine (1-5 mg.) have been found to have no significant hypotensive action in the normal dog (1). When morphine is administered prior to hemorrhage in normal dogs and in dogs during early or impending shock following acute massive hemorrhage, there are no immediate vasodepressor responses demonstrable (2, 3). Systematic investigation has not been made concerning the effects of morphine during the progressive development of a state of shock which becomes increasingly refractory to blood replacement. The present report deals with the vascular responses to morphine when the agent is given during the appearance of the so-called 'irreversible state' of shock in dogs subjected to graded hemorrhage.

Recent studies based on direct observations of the omental capillary bed (4) and bioassay of blood samples (5, 6) have indicated that the syndrome following prolonged hemorrhagic hypotension is divided temporally into three phases: 1) a hyperreactive period, with greatly heightened peripheral vasomotor reactivity, the presence of blood-borne vasoexcitor materials (VEM) and marked vasoconstriction of larger blood vessels; 2) a transitional period, in which the augmented vascular reactivity becomes less pronounced and the blood contains both vasoexcitor and vasodepressor materials (VDM); and 3) a hyporeactive period, characterized by diminished or absent vasomotor activity, vasodilatation, and the predominance of vasodepressor substances in the blood.

After restoring normotensive levels by transfusion during the hyporeactive stage,

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the muscular vessels become only partially narrowed. The blood flow progressively improves but remains sluggish on the venous side of the circulation. Arteriolar and precapillary vasomotion does not reappear, however, and the epinephrine reactivity of contractile vessels increases but seldom returns to normal levels. Approximately 90 per cent of these animals succumb within 3 to 10 hours after completion of the transfusion, with a gradual fall in blood pressures and progressive return of peripheral vascular dysfunction. The influence of morphine and certain other agents were determined with specific reference to each of these categories.

Other investigators have injected morphine prior to bleeding (2), or at the end of one to three hours of moderate hypotension (3). In the latter instances, peripheral vasomotor reactivity was presumably still in the hyperreactive period described above, or in the transitional phase. It has been our experience that during this period the animal is still capable of handling vasodepressor substances, in contrast to their inability to do so during the subsequent vasodepressor stage of the syndrome (6). Moreover, morphine administration during the period subsequent to transfusion closely duplicates the situation in clinical shock when this agent is frequently given post-operatively to patients who have been in shock and transfused.

For this reason, most of the experiments reported here deal with the vascular effects of morphine injected intravenously after the prolonged hypotensive period has been terminated by whole blood transfusion. Post-transfusion blood pressures at the time morphine was administered were essentially similar to those of the control unbled animals. This procedure permitted a comparison of the effects of morphine on animals in the control and in the experimental group with comparable blood pressure ranges.

METHODS

Hemorrhagic shock was induced in dogs in which the omentum was exteriorized for microscopic study. One group of dogs were anesthetized with morphine sulfate, 2 mg. intravenously or 12 mg. subcutaneously per kg. of body weight. Such doses produce a euphoric state in the dog which does not actually represent a state of true anesthesia. These animals were compared to a control group receiving no general anesthesia. In both groups of animals local procaine anesthesia was employed for cannulating the femoral artery and for exteriorizing the omentum. Also included in the present report are several animals anesthetized with cyclopropane and subjected to hemorrhagic shock. In most instances the omentum was exteriorized and kept both warm and moist by a continuous irrigation with Ringer-gelatin maintained at body temperature. This technique has been described in detail in a previous publication (4). Mean blood pressure was recorded continuously by a mercury manometer from the femoral artery. Morphine was administered intravenously (2-6 mg/kg.) during the shock syndrome. On a second group of hemorrhaged animals, morphine was given intravenously following the restoration of normal blood pressure ranges by blood transfusion at the onset of the hyporeactive state.

RESULTS

Unhemorrhaged Animals

Local anesthesia. Fifteen animals (8-15 kg. in weight) were prepared by using a 1.0 per cent procaine solution to produce abdominal field block and the omentum exteriorized for microscopic study. No other medication was given. Blood flow was found to be rapid throughout the arteriolar and venular systems. Spontaneous vasomotion of the terminal arterioles and precapillary sphincters was active and was predominantly in the 'closed' or constricted phase. Capillary blood flow was inter-

mittent and asynchronous. The threshold response of the terminal smooth muscle components of the bed to topically applied epinephrine occurred with concentrations of one part in five to ten million. The arterial blood pressure averages 128 mm. Hg \pm 10 mm.

Morphine anesthesia. Morphine sulfate, 2.0 mg/kg., was slowly injected intravenously (over a 15–20-second period) into 14 dogs. An equal number were given 12 mg. morphine/kg. subcutaneously. The omentum was then exposed following local infiltration with procaine and examined under the microscope.

There were no pronounced differences in the peripheral circulation of the omentum of these animals as compared to that of the controls (table 1). Spontaneous vasomotor activity was in general as frequent and of a duration similar to that in the unmorphinized dogs. Arteriolar tone and peripheral blood flow was comparable in both groups. The epinephrine reactivity of the terminal arterioles and precapillaries in animals receiving morphine was slightly below those of the control dogs (table 1).

TABLE 1. OMENTAL CIRCULATION IN CONTROL DOGS BEFORE BLEEDING

ANESTHESIA	NO. OF DOGS	B.P. MM. Hg	BLOOD FLOW	VASOMOTION	TONE OF VESSELS	EPINEPHRINE REACTIVITY ²
None ¹	15	128	Intermittent, rapid	Prominent	Arterioles and venules narrow	1:8,000,000
Morphine sulfate (2-12 mg/kg.)	28	120	Intermittent, rapid	Slightly exaggerated	Arterioles narrow; venules slightly dilated	1:6,000,000

¹ Omentum exposed by abdominal field block with 1% procaine. ² Topical application of epinephrine—minimal effective concentration.

Hemorrhagic Shock

Morphine prior to blood infusion. A series of 7 dogs were bled to shock levels and morphine was administered during different stages of shock (table 2). During the hyperreactive stage, morphine had no vasodepressor action (*dog 154*—local procaine anesthesia, and *dog 36*—morphine sulfate—12 mg/kg.). One animal (*dog 153*—cyclopropane) was given morphine during the transitional stage just before the peripheral blood vessels became hyporeactive. The blood pressure fell slightly for a short period and then returned to its original level. Four dogs (*nos. 100, 101*—morphine sulfate—6 mg/kg.; *no. 23*—morphine sulfate—2 mg/kg.; *no. 146*—no general anesthesia) were given morphine during the hyporeactive stage of shock and in 3 of these (*dogs 100, 101, 146*) the blood pressure fell precipitously, necessitating immediate transfusion.

Morphine following infusion of blood. Eleven dogs anesthetized with morphine sulfate (2 mg. intravenously or 12 mg. subcutaneously/kg. body weight) and two dogs receiving no general anesthesia were bled by graded hemorrhage until the vascular bed in the omentum had become hyporeactive (in two of the dogs, the hyporeactive stage was determined by assay of the blood). The blood pressure in these animals was on the average from 30 to 40 mm. Hg. The reactivity of the terminal arterioles to epinephrine was in the range of one part in 500,000 as compared with con-

trol responses to one part in 8 to 10,000,000 of epinephrine. Vasomotion was depressed and often absent. Blood flow through the collecting venules and the larger veins (100–150 μ .) was sluggish. The animals were then infused with all of the blood previously withdrawn. The blood pressure in all cases but one (*dog 99*) returned to the 100 to 120 mm. Hg range. With the restored blood volume, mechanical speeding up of blood flow through the capillary bed occurred. The terminal arterioles underwent a moderate narrowing. The responsiveness of the muscular vessels to epinephrine rose, but did not regain control values. Vasomotion was only slightly influenced, if at all, usually remaining depressed.

TABLE 2. MORPHINE ADMINISTERED DURING SHOCKED STATE

DOG	ANESTH.	STAGE OF SHOCK		MOR- PHINE DOSE ¹ mg/kg.	EFFECTS		REMARKS
		B.P. \times min.	Omentum		Blood Pressure	Omentum	
154	None	<80 mm. \times 30	Hyper-reactive	2	No change	Unaffected	Recovered spontaneously
36	M.S. ²	<60 mm. \times 120	Hyper-reactive	3	No change	Unaffected	Reversible by infusion
153	Cyclop.	<80 mm. \times 90	Transitional	2	Transient fall to 60 mm.	Flow slowed	Reversible by infusion
100	M.S.	<40 mm. \times 90	Hyporeactive	3	Fell to 25 mm.	Stagnant	Required infusion to halt circulatory depression
101	M.S.	<40 mm. \times 90		3	Fell to 20 mm.		Died despite infusion of blood
23	M.S.	<60 mm. \times 120	Hyporeactive	1	Fell to 40 mm.	Slowed, arterioles dilated	Irreversible to infusion
146	M.S.	<60 mm. \times 180	Hyporeactive	2	Fell to <20 mm.	Stagnant	Died in 3 hours despite large blood infusion

¹ Intravenously administered. ² M.S. = morphine sulfate.

At this stage, morphine sulfate was given to these animals. In all but two cases (table 3, *dogs 19, 72*), in contrast to the absence in unbled animals of any observed effect of such doses of the drug, the blood pressure fell to subnormal levels (a drop of 40–50 mm. Hg) within 3 to 15 minutes. It then continued to drop more slowly from these values. Concomitant with the fall in blood pressure, the arterioles and pre-capillaries in the omentum became dilated. The blood flow through the capillary bed, especially on the venous side, became sluggish. The threshold response of the muscular vessels to epinephrine rapidly fell to the depressed values present during the hyporeactive stage of shock. Only one of the affected animals (*dog 68*) showed a spontaneous recovery from this condition, returning to a blood pressure of 85 mm. Hg within 45 minutes. In the remaining 9 cases the hypotension persisted and it was found necessary to transfuse the dogs in order to avoid a rapid exitus. Four of the dogs died despite repeated transfusion therapy at this stage.

DISCUSSION

It has been shown previously that morphinized dogs (2-6 mg/kg.) developed a more pronounced depression of the peripheral vascular system in the terminal stage of hemorrhagic shock than non-morphinized control animals (7). However, no major

TABLE 3. MORPHINE ADMINISTERED TO SHOCKED DOGS AFTER BLOOD INFUSION DURING HYPOREACTIVE STAGE

DOG	INITIAL DOSE M.S. ¹	CONDITION OF ANIMAL				MORPHINE DOSE ¹	EFFECTS		REMARKS
		Pre-infusion ² Blood Pressure	Post-infusion		Blood Pressure		Omentum		
			Blood pressure	Omentum					
	per kg.	mm. Hg X hrs.	mm. Hg X hrs.			mg/ kg.	mm. Hg		
19	2 mg.	<70 X 3.0	110 X 3.0	Normal reactivity		0.4	No change	No change	Animal reversible
68	12 mg.	<70 X 4.5	100 X 0.5	Normal reactivity		1.0	Fell to 75	Slowing of flow	B.P. and flow restored in 25 min.
111	None	<40 X 2.5	110 X 0.5	Arterioles tonic		2.0	Fell to 70	Flow stagnant	B.P. and flow restored only by infusion of 150 cc. blood
107	None	<40 X 1.5	100 X 0.1	Hyporeactive		2.0	Fell to 60	Flow sluggish	Died in 20 min. despite infusion
99	2 mg.	<50 X 4.0	50 X 0.5	Hyporeactive		2.0	Fell below 20	Complete cessation of flow	Died in 10 min. despite infusion
38	12 mg.	<60 X 4.5	90 X 0.75	Reactivity somewhat improved		2.7	Fell to 70	Flow slowed especially in veins	Died 3 hrs. later
56	12 mg.	<60 X 3.5	100 X 0.5	Hyporeactive		3.6	Fell to 70	Dilatation of arterioles	B.P. and flow restored by infusion of saline 10 cc/kg.
47	12 mg.	<50 X 3.0	100 X 1.0	Tone of vessels somewhat improved		4.2	Fell to 80	Pronounced slowing of flow	B.P. and flow slowly restored by repeated infusions
72	12 mg.	<60 X 6.0	110 X 2.0			4.8	Slight fall		No change for 1 hr. then sudden collapse
57	12 mg.	<50 X 3.0	115 X 0.5	Circulation improved, reactivity still sub-normal		6.0	Fell to 70	Temporary slowing of flow	B.P. and flow restored by saline-pitressin infusion
30	12 mg.	<70 X 5.5	100 X 2.0	Circulation almost normal		6.0	Fell to 60	Arterioles dilated	B.P. and flow restored by infusion of saline 5 cc/kg.
58	12 mg.	<50 X 5.0	105 X 0.5	Circulation improved, reactivity still sub-normal		9.0	Fell to 30	Stagnant	B.P. and flow restored by saline-albumin infusion 6 cc/kg.
33	12 mg.	<50 X 4.0	100 X 0.2			10.0	Fell to 65		Died in 1.5 hrs. despite infusion

¹ M.S. = morphine sulfate.

² Omentum in hyporeactive state.

³ Intravenously administered.

differences were observed in the depth or duration of the hypotension, or in the relative blood-loss necessary to produce eventual irreversibility to whole blood restoration. Similarly, both in dogs with no general anesthesia and dogs under morphine, the hyperreactive phase of early hemorrhage was of the same general range and duration. These findings, together with the comparatively unimpaired vascular reactivity found in the morphinized unbled dogs in this study, suggest that the periph-

eral vasomotor mechanisms prior to and during early blood-loss can compensate for any vasodepressor properties of morphine in doses of 1 to 3 mg/kg.

The effects of morphine during the subsequent course of the hemorrhagic shock syndrome appear to depend upon the duration of profound hypotension and the dose of morphine employed. Thus, the three animals receiving 2 to 3 mg. of morphine per kg. of body weight prior to the development of hyporeactivity in the omentum showed either a negligible or transient fall in blood pressure and slowing of omental circulation. This is in agreement with the observations of Reed and co-workers who likewise administered morphine early in hemorrhagic hypotension (3).

Morphine administered during the hyporeactive stage of shock was followed by a profound and sustained fall in blood pressure together with a considerable slowing of the peripheral circulation. Morphine administered to dogs which had been transfused during the hyporeactive stage of shock likewise produced a rapid deterioration of the peripheral circulation and a significant lowering of the blood pressure. It is evident that animals which have already suffered peripheral vascular damage are especially susceptible to the vasodepressor action of morphine. This is of considerable significance in the use of morphine as an analgesic agent in the immediate post-hypotensive period. In dogs subjected to hemorrhage such medication is definitely contraindicated.

The precise mechanism whereby morphine acts to depress the peripheral circulation is not clear. The presence of considerable amounts of a vasodepressor principle VDM in the blood during the latter stages of hemorrhagic shock suggests that morphine may intensify the decompensatory action of this principle on the peripheral vessels. Whether the effect is myotoxic or is mediated through an interference with the sympathetic vasoconstrictor impulses in the terminal vessels remains to be determined.

SUMMARY

Based on changes in blood pressure and direct visual observation of omental peripheral blood vessels, usual doses of morphine (2-12 mg/kg.) in unhemorrhaged dogs produce no notable disturbances.

Morphine (2-3 mg/kg.), when given early in hemorrhagic shock, is followed by no sustained vascular depression. Morphine (0.4 to 10 mg/kg.) administered during latter stages of hemorrhagic shock usually lowers the blood pressure and depresses the peripheral circulation for a variable period of time. The vasodepressor effect does not appear to be directly related to the dosage administered.

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EFFECT OF ALTITUDE ON RESPIRATORY FLOW PATTERNS¹

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STUDIES of breath velocity and the timing of the various phases of the breath were begun by us in connection with the physiological assessment of high altitude oxygen equipment.² The need for fundamental data regarding the behavior of breathing in response to physical changes in the atmosphere is apparent from the lack of treatment of this subject in physiology texts and the preoccupation of physiologists with the effects of variations in the partial pressure of oxygen and carbon dioxide.

Fleisch (1, 2) and Bretschger (3) made breath velocity analyses, demonstrating some of the neuromuscular and physical factors which interact in pulmonary ventilation. Gukelberger (4, 5) made particularly sensitive analyses of normal breath velocity patterns in terms of acceleration values, but without attempting physical analysis of the non-physiological components. Silverman *et al.* (6, 7) studied the effects of various pulmonary disease conditions on the breath velocity pattern but did not consider the methods of pattern analysis which had been made by Gukelberger, probably because of the isolation of European literature occasioned by World War II. Silverman's analysis did not consider physical changes in the inspired air as used in palliative and therapeutic helium treatment. Dean and Visscher (8), on the other hand, did not use the pneumotachographic method in analyzing the mechanism of helium-oxygen administration in pulmonary obstruction therapy. Thus, despite a few observations by Pappenheimer and Lilly (9) in 1943 and the 1926 work of Fleisch (2) on breath mechanics at altitude (which, incidentally, demonstrated certain of the phenomena reported here and indirectly supports our hypotheses) the essential effects on breathing of the physical factors introduced by the atmosphere, have not been recognized.

While our studies originated in response to the need for flow patterns which would facilitate the efficient and safe design of oxygen regulators and mask valves, the studies soon led into considerations of the relations between various phases of the breath and their bearing on the efficiency of pulmonary functions affecting gaseous exchange in the pulmonary system. The data presented are intended to show primarily the norms of breathing behavior under rather special conditions, and secondarily, the physical basis for the responses of the pulmonary ventilation to breathing at high altitude, and their possible application in assays of pulmonary function.

Early observations (10) on respiratory behavior made with closed circuit oxygen

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regenerating systems indicated to us that ascent to 30,000 feet simulated altitude induced a decrease in respiratory rate and a compensatory increase in tidal volume resulting in relative constancy of minute volume. It is probable that the attainment of this relation was fortuitous and due to the particular experimental setup, but the general effect of altered breath timing has been substantiated in our later work. Since full oxygenation of the blood was assured at altitude by the conditions of the experiments, it was deduced that, in contradiction to all but Barach's (11) hypothesis, the factor which brought about these changes was gas density or a related physical

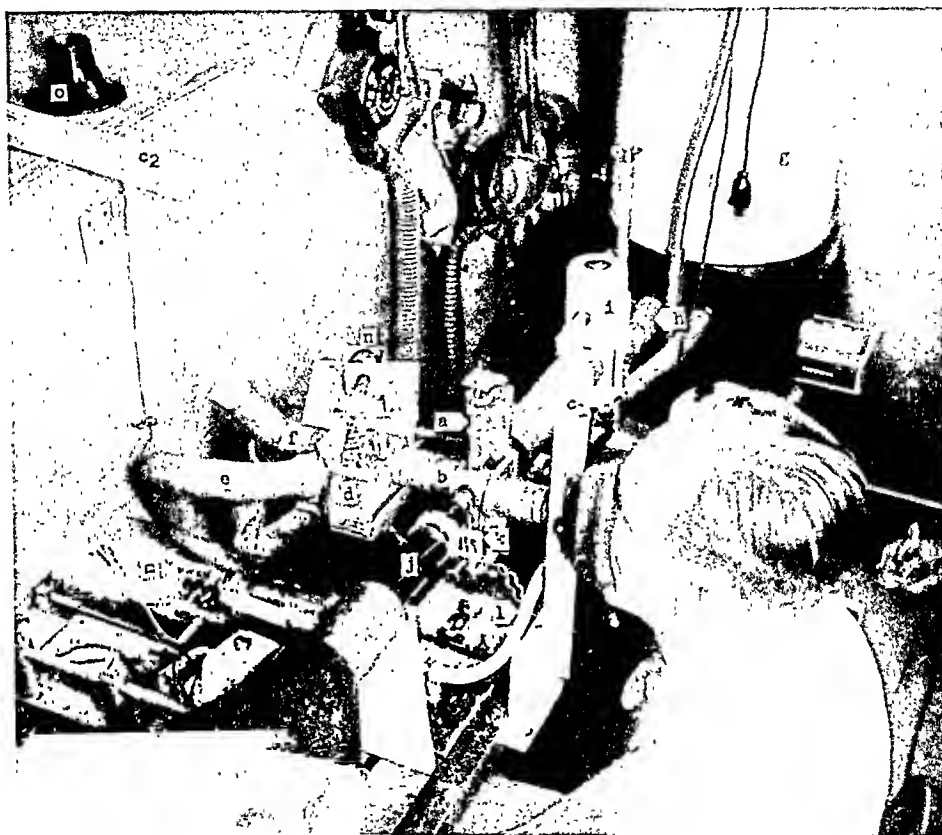


Fig. 1. FLOW MEASURING INSTRUMENT AND NECESSARY APPARATUS: *a*, wire suspension; *b* breathing tube; *c*₂ and *c*₃, bracing; *d*, valve housing; *e*, expiratory tube; *f*, inspiratory tube with outlet; *g*, spirometer; *h*, iris diaphragm resistance on inspiratory tube; *i*, mercury arc lamp; *j*, projection system to camera (not shown); *k*, heater unit to prevent condensation on window; *l*, pressure tube to *m*, relay switch for activating *n*, spring opposed solenoid; *o*, Borda mouthpiece to replace valve when breathing air.

property. On the strength of these observations a series of experiments was set up to measure quantitatively the changes induced in the timing of the phases of the breath cycle and in breath velocities.

APPARATUS AND TEST CONDITIONS

The desirability of influencing breath velocity as little as possible by frictional resistance in the air stream (which would also respond to changes in density) led to the adaptation of the flowmeter design used by Silverman (12) modified in certain respects as described in (13) and shown in figure 1. Essentially, the instrument

records photographically the deflection of the shadow of a fine wire stretched through the breath stream. Since a record of both expiratory and inspiratory flows was desired, it was necessary to add dead space in excess of that of the mask. Data presented by Stannard and Russ (14) on the effects of added dead space on the ventilation indicate that a gain of about 16 per cent in the tidal volume results from a dead space of about 150 cc. (slightly more than the volume of the flowmeter). They found no consistent influence on the breathing rate.

A double butterfly valve (13) actuated indirectly by the breath pressure was used to insure proper circulation of the respired gases from the distal end of the flowmeter. It offered minimal resistance to all flows encountered. A backflow, of low order as compared to sensitive rubber valves (12) used for the same purposes, was incurred by the unavoidable delay of the tripping mechanism. In common with other valves, efficiency rises with the respiratory rate and breath velocity.

The valve openings were connected distally to a closed oxygen circuit of 1.25 inch i.d. rubber tubing, disposed in such a manner as to avoid sharp turns and connecting with a recording spirometer which had a CO₂ absorbing filter in the expiratory duct and an adjustable iris diaphragm for adjustment of resistance in the inspiratory duct.

Measurements of the pressure drop from mask to spirometer with constant velocity, using a rotameter in place of the subject, were made with a slant manometer. The pressure drops at various airflows were as follows:

L/MIN.	'EXPIRATION' PRESSURE IN MM. H ₂ O	'INSPIRATION' SUCTION IN MM. H ₂ O
20	= 1.4	= 1.1
40	= 2.2	
50	= 3.9	= 2.0
70	= 5.8	= 3.0
80	= 7.5	
100	= 9.0	= 4.0

Measurements with oxygen instead of air gave essentially equivalent values, indicating that orifices in this system were large enough to have only a negligible influence at the velocities encountered.

The restriction caused by a standard setting of the iris diaphragm resulted in a 100-fold increase in inspiratory suction at peak flows, as shown by the following data, which suggest a slight difference between air and oxygen in this respect:

L/MIN.	'INSPIRATION' (RESTRICTED) AIR SUCTION IN MM. H ₂ O	'INSPIRATION' (RESTRICTED) OXYGEN SUCTION IN MM. H ₂ O
20	= 15	= 20
30	= 38	= 45
40	= 83	
50	= 140	= 154
60	= 212	= 227
70	= 303	= 318

A comparison of measurements of breath patterns obtained when breathing air through the flowmeter alone and through the flowmeter with the added valve and spirometer circuit were made in order to assess the alterations induced by these

factors of resistance. As indicated above, the flow pressures due to unrestricted flows of the order encountered in normal breathing (up to 70 l/min.) were very low. Such pressure changes are minor in comparison with other uncontrolled factors affecting breath velocity, and hence do not correlate with respiratory responses. However, paired experiments on five subjects showed that the addition of the valve and spirometer caused a statistically significant 31 per cent increase in the length of the respiratory cycle; a 10 per cent decrease in the maximum velocity of inspiration and a 32 per cent increased rate of termination of inspiration (Ipd) were not statistically significant.

It was fully appreciated that so-called 'normal' conditions of breathing could not be expected, even though further precautions might be taken, since most individuals change their breathing response when confronted with a mask and respiratory measuring devices. It is felt that a knowledge of these changes induced by the experimental procedure will assist in the interpretation of the data.

SUBJECTS AND TEST PROCEDURE

Subjects for this study were 32 male volunteers, previously indoctrinated for altitude work by the Naval Medical Research Institute staff. Their ages were: 6 at 17 years; 20 at 18 years; 1 at 19 years; 1 at 26 years; 1 at 29 years; 2 at 30 years, and 1 at 36 years. In general, they were not apprehensive, since the whole test routine was run off at ground level before the altitude run was made. A few experienced some difficulty on descent due to ear block, but this was subsequent to the tests. Only one man reported gas pains at altitude but was able to complete the tests.

A concise outline of procedure in non-technical words was given before the test in order to obtain proper cooperation in maintaining the seal of the mask and in following the variations of the procedure. It was emphasized that the subject was breathing full oxygen at all times. The breathing circuit was flushed out with O₂ before each recording.

The subjects sat with chest erect so that the face rested firmly in the inflated rim of the mask. The principal opening of the mask was of the same bore as the flowmeter and was placed directly before the mouth and nares. The subject was instructed to breathe as naturally as possible both in the trial period and during the recording. After the test was completed it was ascertained whether or not the subject had resorted to mouth breathing.

The record was preceded by a baseline recording which required the subject to hold his breath momentarily, and this was followed by a series of 8 to 10 breaths before further recording. At the end of this interval, inspection of the spirogram indicated to the operator whether or not a characteristic record could be taken.

A record of at least 4 consecutive breaths was taken with the subject at rest. This was followed by a record during an arbitrary constriction of the inspiratory path by means of the iris diaphragm. The subject was then exercised by making 15 deep knee bends from a standing position at a rate of his own choosing, but not including perceptible pauses. The subject steadied himself, if necessary, by one hand.

Following the exercise the subject breathed through the flowmeter, and a record was made without intervening breath holding in order to obtain the most enhanced velocities subsequent to the exercise. A second bout of exercise, but limited to 10 deep knee bends, was performed by the subject prior to breathing on the restricted circuit. Pulse rates were recorded after both bouts of exercise to indicate whether or not equivalent stress had been effected. The pulse rates approximated 125 to 130/minute and indicate a work equivalent to about 600 kg.M/min. (5).

This routine was repeated precisely shortly after attaining an altitude of 30,000 feet at a rate of 3000 feet per minute. The subject was provided with full oxygen



Fig. 2. ONE RESPIRATION from sample tracing showing most of the points discussed. Subject at rest breathing O_2 at 30,000 ft. simulated altitude. Time signals at bottom of record in seconds; horizontal lines 5 mm. apart. Ink spots show approximate location of numbered reference points: 1 to 2 = duration of inspiration (*Is*); 2 to 3 = duration of expiration (*Es*); 3 to 4 = duration of expiratory pause (*Eps*); 1 to 4 = duration of total breath (*Bs*); 5 = inspiratory maximum deflection (87 l/min.) (*Imx*); 8 = expiratory maximum deflection (132 liters per minute) (*Emx*); 1 to 5 = inspiratory acceleration (*Ia*); 2 to 7 = expiratory acceleration (*Ea*); 5 to 6 = inspiratory plateau (*Ip*); 7 to 8 = expiratory plateau (*Ep*); 6 to 2 = inspiratory drop (*Id*); 8 to 3 = expiratory drop (*Ed*).

during ascent and at altitude, and changed from mask to flowmeter and back while holding the breath momentarily.

Velocity calibrations were made after each run and applied to the individual records.

METHOD OF ANALYSIS OF THE DATA

The primary data obtained from these tests were the breathing pattern photographically registered from the wire shadow and the displacements of the spirometer in the breathing circuit. A sample tracing is shown in figure 2.

The velocity pattern was resolved into the following items which appeared to be phenomena common to each breath, except as noted:

1. *Inspiration*. The time in seconds from the rise from the resting position of the wire to the return to that level, (*Is*).

2. *Inspiratory-expiratory pause.* The time in seconds from end of 1 to the crossing of the wire in the opposite direction from the resting position, (Ips). Absent in most individuals, especially after exercise.

3. *Expiration.* The time in seconds from the end of 2 to the return of the wire to the resting position, (Es).

4. *Expiratory-inspiratory pause.* The time in seconds from 3 to the rise from the resting position of the wire denoting the next inspiration, (Eps). Many individuals do not have it at ground level.

5. *Total time for one breath cycle in seconds,* (Bs).

6. *Rate of respiration,* (60/Bs).

7. *Inspiratory 'peak' velocity.* The velocity attained at the beginning of the plateau phase in inspiration in liters per minute (Imf).

8. *Expiratory 'peak' velocity.* As in 7 but for the expiratory phase.

Both the inspiratory and expiratory velocity records were further analyzed into three parts: acceleration, plateau and deceleration, as listed below. In our experience the plateau was almost always somewhat rounded, and in approximately three fourths of the cases the plateau had a definite negative slope. The remaining cases were level or had a positive slope.

9. *Inspiratory acceleration.* Rate of increase in velocity in liters/min/sec., (Ia). In tests with inspiratory restriction this was used only in calculating breath volume, since an initial backflow allowed a momentary 'spike' to develop.

10. *Inspiratory plateau.* Magnitude and sign of slope of plateau in liters/min/sec., (Ip).

11. *Inspiratory deceleration.* Rate of decrease in velocity in liters/min/sec., (Id).

12. *Expiratory acceleration.* As in 9 (Ea).

13. *Expiratory plateau.* As in 10 (Ep).

14. *Expiratory deceleration.* As in 11 (Ed).

From the spirometer tracings the following additional measurements were made:

15. *Tidal volume in liters,* (Tv).

16. *Minute volume.* Liters of gas ventilated through the lungs. Calculated from 15 and 6 (Mv).

The photographic records were provided with time lines at 1/240 and 1-second intervals. It was customary to measure time intervals from the record by means of a proportionally adjustable rule in order to allow paper speed differences to be equated. The rule was marked in tenths of a second and allowed estimation of .05 second. The records were run at such a rate as to allow reasonably accurate measurements to be made with this method.

Due to mechanical limitations of stretched or suspended wires, as in this flow-meter, the zero positions following inspiration and expiration were different. The damping of this particular instrument caused the variations in absolute position to be more pronounced than would be the case if free oscillation had been permitted. However, this deviation was known and accounted for in estimating pauses, which appear in the record as level stretches in the pattern in the immediate vicinity of the arbitrary zero position. The maximum zero displacement found in our instrument was ± 3 mm. from the resting position at an optical magnification of about 250 diameters. This corresponds to an apparent velocity of about 12 liters per minute,

TABLE 1. RESPIRATORY PATTERN DATA AT GROUND LEVEL AND ALTITUDE, WITH AND WITHOUT EXERCISE

	REST ¹		EXERCISE ²	
	Ground Level	30,000 Feet	Ground Level	30,000 Feet
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
Inspiration	1.430±.298	1.440±.291	1.298±.252	1.293±.294
Insp. pause	.045±.089	.072±.095	.002±.009	.087±.093
Expiration	1.622±.418	1.490±.373	1.333±.262	1.363±.392
Exp. pause	.122±.166	.320±.241	.023±.067	.088±.114
Total	3.217±.762	3.285±.667	2.655±.510	2.831±.793
	<i>no.</i>	<i>no.</i>	<i>no.</i>	<i>no.</i>
Respirations per minute	19.69±4.13 (11.3-27.9)	19.27±4.38 (11.8-30.3)	23.48±5.12 (18.3-34.7)	22.83±6.51 (14.0-35.3)
	<i>l/min.</i>	<i>l/min.</i>	<i>l/min.</i>	<i>l/min.</i>
Inspiratory peak flow	43.0±9.5 (21-66)	43.0±12.4 (12-97)	47.9±11.1 (32-74)	59.6±27.7 (18-117)
Expiratory peak flow	48.5±12.7 (19-71)	56.9±19.9 (17-134)	54.5±21.8 (16-108)	80.8±41.9 (18-203)
	<i>liters/min/sec.</i>			
Inspiratory Acceleration	112.6±32.9	121.9±52.7	138.1±49.6	271.8±233.6
Inspiratory Plateau	-12.6±17.0	-16.5±20.2	-12.9±17.6	-6.0±25.2
Inspiratory Deceleration	-98.5±37.1	-106.4±46.1	-126.8±46.2	-197.5±149.1
Expiratory Acceleration	105.4±34.4	132.7±44.6	145.8±65.7	279.3±201.0
Expiratory Plateau	-24.0±26.6	-32.3±32.0	-37.1±22.2	-48.1±60.4
Expiratory Deceleration	-65.9±31.6	-74.9±52.9	-95.2±28.7	-193.6±210.9
	<i>liters</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>
Tidal Volume	0.773±0.156 (.52-1.13)	0.696±0.136 (.49-1.10)	0.923±0.304 (.50-1.76)	1.044±0.222 (.66-1.43)
Minute Volume	14.86±3.29 (9.6-25.8)	13.04±2.37 (9.2-19.5)	20.49±4.76 (14.4-28.3)	23.73±9.40 (15.3-50.5)

¹ Values are means of 32 subjects' averages, 2 to 6 breaths per subject, and std. dev. ² Values are means of 12 subjects' averages, 3 breaths per subject, and std. dev. Values in parentheses are ranges. Negative sign denotes deceleration.

but lies in the lower extrapolated portion of the calibration. This action of the wire did not interfere with the accuracy of measurement of the velocities given in these data since due allowance was made for the proper zero positions.

RESULTS

The data compiled from the series of tests on Navy volunteers are shown in tables 1 and 2. The means shown are the means of the mean values for individuals,

weighted equally and thus give an indication of the variation in the population which we studied.

TABLE 2. RESPIRATORY PATTERN DATA AT GROUND LEVEL AND ALTITUDE WITH AND WITHOUT EXERCISE, WITH ADDED INSPIRATORY RESISTANCE

	REST		EXERCISE	
	Ground Level	30,000 Feet	Ground Level	30,000 feet
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
Inspiration	1.422±.288	1.405±.304	1.339±.226	1.231±.260
Insp. pause	.040±.080	.081±.084	.004±.014	.038±.064
Expiration	1.448±.287	1.349±.280	1.263±.232	1.298±.387
Exp. pause	.057±.091	.278±.259	.004±.014	.050±.072
Total	2.968±.627	3.114±.734	2.610±.424	2.618±.670
	<i>no.</i>	<i>no.</i>	<i>no.</i>	<i>no.</i>
Respirations per minute	21.16±4.87 (15.9-28.3)	20.39±5.28 (13.9-28.7)	23.62±4.26 (18.5-30.8)	24.23±11.19 (14.4-35.9)
	<i>l/min.</i>	<i>l/min.</i>	<i>l/min.</i>	<i>l/min.</i>
Inspiratory peak flow	34.6±8.7 (20-66)	34.8±12.4 (13-63)	44.8±10.7 (26-72)	59.1±18.7 (11-94)
Expiratory peak flow	44.9±13.7 (26-75)	46.5±17.0 (9-85)	63.7±25.6 (27-118)	85.2±30.4 (25-144)
	<i>liters/min./sec.</i>			
Inspiratory Acceleration	133.8±17.8	148.9±75.3	210.3±77.8	388.7±685.6
Inspiratory Plateau	-16.8±8.8	-1.0±14.9	-14.1±18.6	-4.9±13.0
Inspiratory Deceleration	-98.8±45.7	-114.4±70.6	-129.1±45.7	-209.8±106.7
Expiratory Acceleration	108.3±49.7	154.0±55.9	167.8±79.9	299.8±209.2
Expiratory Plateau	-22.6±29.9	39.8±24.0	-40.1±28.0	-70.6±52.7
Expiratory Deceleration	-79.4±37.4	-71.2±64.3	-119.0±79.9	-259.6±224.0
	<i>liters</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>
Tidal Volume	0.766±0.133 (0.50-0.94)	0.709±0.171 (0.43-0.97)	0.931±0.219 (0.56-1.34)	1.059±0.257 (0.77-1.44)
Minute Volume	15.92±3.96 (12.3-26.1)	14.11±3.43 (7.0-19.8)	21.47±4.06 (16.1-29.7)	25.55±8.15 (10.4-38.7)

Values are means of 12 subjects' averages, 3 breaths per subject, and std. dev. Values in parentheses are ranges. Negative sign denotes deceleration.

Figure 3 presents graphically the salient points of the tables in order to show the inter-relation of the data for any one condition and also to provide a ready comparison of the effects of altering the several conditions of altitude and exercise. It

must be recognized that the flow patterns as shown in figure 3 are derived in all instances from rounded curves as in figure 2. It is felt that the beginning of inspiration is the logical point of origin for these diagrams, and this presentation tends to distinguish curves having even small timing differences better than would the use of the inspiratory-expiratory crossover point as the point of coincidence of the several types of pattern (15).

DISCUSSION

The following statistical treatment of the data is made on the basis of paired values from each subject. The mean of the differences for the various subjects is

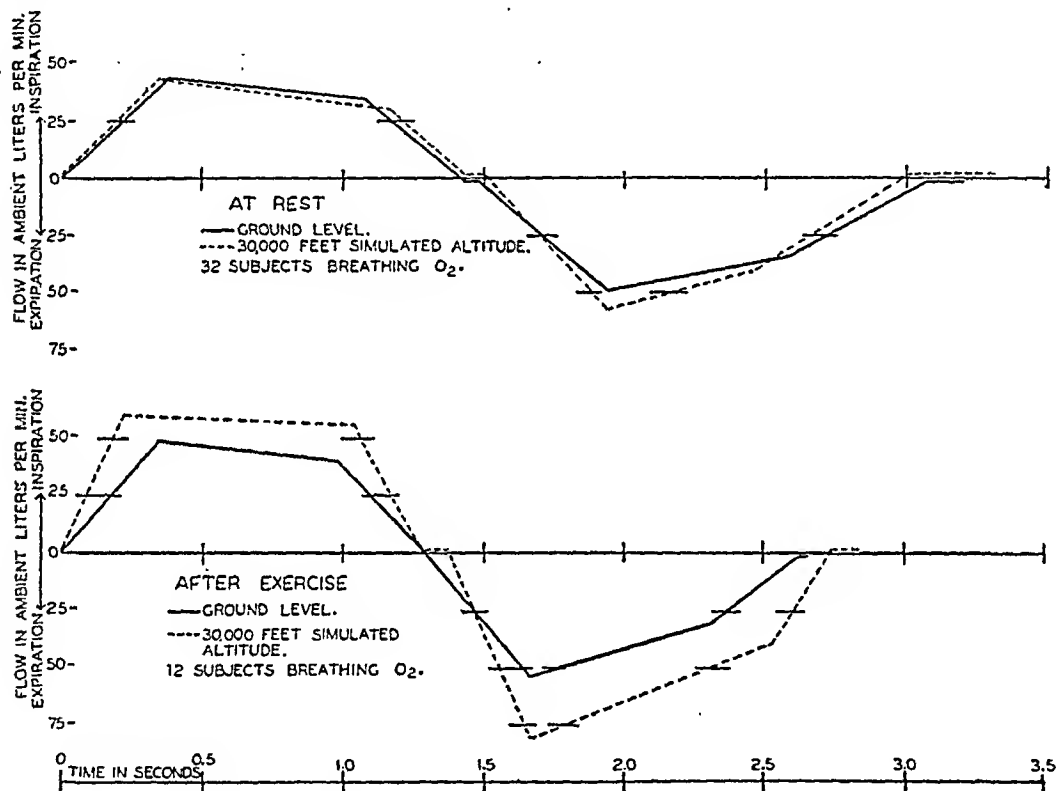


Fig. 3. BREATHING PATTERNS at ground level and at altitude.

given below, accompanied by a statistical evaluation in terms of standard deviation of the differences, standard deviation of the mean, and the probability of the difference being due to chance, and the ratio of the difference to the control mean in percentage. Comparisons showing no statistical significance for the difference are not given since they may be obtained from the tables.

Effect of Altitude on Respiratory Pattern. In the resting condition without added inspiratory resistance, ascent to 30,000 feet alters:

Eps, $+ .20$ sec., $s = .24$, $\bar{s} = .04$, $P = .001$, $+16.7\%$

Emf, $+8.37$ l/min., $s = 15.30$, $\bar{s} = 2.70$, $P = .001$ to $.01$, $+17.2\%$

Ea, $+27.25$ l/min./sec., $s = 36.46$, $\bar{s} = 6.45$, $P = .001$, $+25.9\%$

Tv, $- .078$ liters, $s = .110$, $\bar{s} = .019$, $P = .001$, -9.9%

Mv, -1.81 liters, $s = 2.23$, $\bar{s} = .39$, $P = .001$, -12.1%

When restriction is added to the inspiratory path at rest, as above, ascent to 30,000 feet alters:

Eps, +.222 sec., $s = .255$, $\bar{s} = .074$, $P = .01$, +369%
 Ip, +15.8 l/min./sec., $s = 12.9$, $\bar{s} = 3.7$, $P = .001$ to $.01$, +92.8%
 Ea, +45.6 l/min./sec., $s = 68.3$, $\bar{s} = 19.7$, $P = .02$ to $.05$, +42.2%

Following exercise, without added restriction, ascent to 30,000 feet alters:

Ips, +.084 sec., $s = .082$, $\bar{s} = .024$, $P = .001$ to $.01$
 Emf, +26.3 l/min., $s = 35.9$, $\bar{s} = 10.4$, $P = .02$ to $.05$, +47.8%
 Ia, +133.7 l/min./sec., $s = 195.3$, $\bar{s} = 56.4$, $P = .02$ to $.05$, +96.8%
 Ea, +133.4 l/min./sec., $s = 178.6$, $\bar{s} = 51.5$, $P = .02$ to $.05$, +91.3%

When resistance is added to the inspiratory path after exercise, ascent to 30,000 feet alters:

Eps, +.046 sec., $s = .069$, $\bar{s} = .020$, $P = .02$ to $.05$
 Imf, +14.3 l/min., $s = 12.7$, $\bar{s} = 3.7$, $P = .001$ to $.01$, +31.8%
 Emf, +21.5 l/min., $s = 21.4$, $\bar{s} = 6.2$, $P = .001$ to $.01$, +33.6%
 Id, -80.7 l/min./sec., $s = 87.7$, $\bar{s} = 25.3$, $P = .001$ to $.01$, -62.5%
 Ea, +132.8 l/min./sec., $s = 186.1$, $\bar{s} = 53.7$, $P = .02$ to $.05$, +79.0%
 Ep, -30.5 l/min./sec., $s = 16.5$, $\bar{s} = 4.8$, $P = .001$, -76.3%
 Ed, -140.6 l/min./sec., $s = 182.8$, $\bar{s} = 52.8$, $P = .02$, -117.6%
 Tv, +.128 liters, $s = .182$, $\bar{s} = .053$, $P = .02$ to $.05$, +13.7%

In no case was there a significant change in average inspiratory or expiratory time, although this may be largely due to the rather marked variation in response. It was further observed that the breath interval, Bs, did not change significantly with altitude even though statistically significant changes in the pauses may have occurred. This also may be due largely to the marked variation. In view of this variation the distribution of the data is of interest. The distribution was found to be normal and, in particular, no bimodal tendencies were observed. This eliminates the possibility that a saltation to both sides of the normal value, still maintaining a stable average, might have been induced by the factor under investigation.

Retesting subjects on a subsequent day, both at ground level and at altitude in the resting condition, showed that statistically significant changes, some negative and most of them positive, occurred in Is, Ips, Bs, Emf, Ea, and Tv due to unknown causes. However, no difference was found in control tests repeated within about one hour. It is noteworthy that despite an increase in ambient volume of backflow at altitude, a decrease in both tidal volume and minute volume occurs at rest, and only a moderate increase occurs after exercise.

Effect of a Standard Exercise on Breathing Pattern. At ground level, without added resistance, the imposition of exercise alters:

Es, -.142 sec., $s = .150$, $\bar{s} = .043$, $P = .001$ to $.01$, -9.6%
 Bs, -.358 sec., $s = .288$, $\bar{s} = .083$, $P = .001$ to $.01$, -11.9%
 Emf, +10.9 l/min., $s = 16.5$, $\bar{s} = 4.8$, $P = .02$ to $.05$, +24.8%
 Ep, -18.2 l/min./sec., $s = 26.3$, $\bar{s} = 7.6$, $P = .02$ to $.05$, -95.8%
 Ed, -29.5 l/min./sec., $s = 29.5$, $\bar{s} = 8.5$, $P = .001$ to $.01$, -44.6%
 Mv, +4.54 liters, $s = 3.85$, $\bar{s} = 1.11$, $P = .001$ to $.01$, +28.4%

At ground level, with added inspiratory resistance the effect of exercise is to alter:

Es, $-.185$ sec., $s = .144$, $\bar{s} = .042$, $P = .001$, -12.8%
 Eps, $-.052$ sec., $s = .081$, $\bar{s} = .023$, $P = .02$ to $.05$, -86.6%
 Bs, $-.358$ sec., $s = .312$, $\bar{s} = .090$, $P = .001$ to $.01$, -12.1%
 Imf, $+10.3$ l/min., $s = 9.1$, $\bar{s} = 2.6$, $P = .001$ to $.01$, $+29.6\%$
 Emf, $+18.8$ l/min., $s = 15.6$, $\bar{s} = 4.5$, $P = .001$ to $.01$, $+41.7\%$
 Id, -30.3 l/min./sec., $s = 32.7$, $\bar{s} = 9.4$, $P = .001$ to $.01$, -30.5%
 Ea, $+59.3$ l/min./sec., $s = 53.7$, $\bar{s} = 15.5$, $P = .001$ to $.01$, $+54.9\%$
 Ed, -39.6 l/min./sec., $s = 40.5$, $\bar{s} = 11.7$, $P = .001$ to $.01$, -50.0%
 Tv, $+165$ liters, $s = 1.66$, $\bar{s} = .048$, $P = .001$ to $.01$, $+21.4\%$
 Mv, $+5.55$ liters, $s = 4.60$, $\bar{s} = 1.33$, $P = .001$ to $.01$, $+34.9\%$

At 30,000 feet, without added resistance, the imposition of exercise alters:

Eps, $-.197$ sec., $s = .210$, $\bar{s} = .061$, $P = .001$ to $.01$, -70.2%
 Imf, $+21.8$ l/min., $s = 24.6$, $\bar{s} = 7.1$, $P = .01$ to $.02$, $+57.3\%$
 Emf, $+38.7$ l/min., $s = 40.0$, $\bar{s} = 11.5$, $P = .001$ to $.01$, $+92.2\%$
 Ea, $+133.6$ l/min./sec., $s = 197.3$, $\bar{s} = 57.0$, $P = .02$ to $.05$, $+91.5\%$
 Tv, $+329$ liters, $s = 1.76$, $\bar{s} = .051$, $P = .001$, $+45.7\%$
 Mv, $+9.94$ liters, $s = 9.33$, $\bar{s} = 2.69$, $P = .001$ to $.01$, $+68.0\%$

Restriction, under conditions of exercise, at 30,000 feet alters:

Is, $-.174$ sec., $s = .246$, $\bar{s} = .071$, $P = .02$ to $.05$, -12.4%
 Eps, $-.228$ sec., $s = .231$, $\bar{s} = .067$, $P = .001$ to $.01$, -81.3%
 Bs, $-.497$ sec., $s = .506$, $\bar{s} = .146$, $P = .001$ to $.01$, -16.0%
 Imf, $+24.3$ l/min., $s = 14.7$, $\bar{s} = 4.3$, $P = .001$, $+69.7\%$
 Emf, $+38.7$ l/min., $s = 20.6$, $\bar{s} = 5.9$, $P = .001$, $+82.3\%$
 Id, -95.3 l/min./sec., $s = 83.1$, $\bar{s} = 24.0$, $P = .001$ to $.01$, -83.5%
 Ea, $+145.8$ l/min./sec., $s = 196.8$, $\bar{s} = 56.8$, $P = .02$ to $.05$, $+94.6\%$
 Ed, -188.4 l/min./sec., $s = 198.1$, $\bar{s} = 57.2$, $P = .001$ to $.01$, -265.3%
 Tv, $+350$ liters, $s = .129$, $\bar{s} = .037$, $P = .001$, $+49.3\%$
 Mv, $+11.44$ liters, $s = 6.49$, $\bar{s} = 1.87$, $P = .001$, $+81.0\%$

These analyses show that the most consistent changes produced by exercise are in the maximum expiratory velocity (Emf) and in the minute-volume (Mv). The only categories in which no significant change is effected in any of the several conditions are the inspiratory pause (Ips) and the inspiratory plateau (Ip). The former being an infrequent phenomenon at best makes this consideration of doubtful significance.

Effect of Restriction of Inspiratory Path on Breathing Pattern. In general restriction doubles the number of categories of significant changes brought about by exercise, and enhances the effect in nearly every category. It similarly can be seen to increase the number of categories of significant changes produced by altitude under conditions of exercise, although the nature of the changes varies between individuals. Difficulties in technique introduced by restriction in the inspiratory line themselves preclude a more rigorous analysis.

General. In view of the uncontrollable factors of motivation and other psychomotor effects incident to the mechanics of the test and chamber operation, repeated tests were not made routinely during the same day. In retrospect, this seems to constitute an omission in the data which might have furnished a clue to effects of the

disturbing factors mentioned above, but in view of the added number of controls demanded and the mass of observations necessary to assess such psychomotor states it was felt that this limitation in procedure was justifiable.

Measurement of the areas delineated by the graphs shown in figure 3 indicates that, in the normal resting condition, both at ground level and at altitude, a greater volume is exhaled than inhaled. The difference is of the order of 13 and 17 per cent, respectively, and appears to be real, even though simplification of the pattern has resulted in arbitrarily reducing the areas from those delineated by the rounded curves of the control records (fig. 2). This apparent paradox may be accounted for on the hypothesis that increases in the volume of inhaled gases caused by heat and water vapor, outweigh the loss of volume due to the respiratory exchange of oxygen and carbon dioxide. Bretschger's (3) data show the reverse relation to hold in his measurements using a pressure drop method of flow measurement. The constant loss of inert gas from the body while breathing with oxygen apparatus probably does not contribute significantly to this effect. Following exercise at ground level no differences in inhaled and exhaled volumes were apparent. This follows, in principle, from considerations of the limitation in effectiveness of the factors listed above, especially since pauses are reduced or eliminated. At altitude, on the other hand, records of breathing after exercise again show a difference between inspired and expired gas volumes concomitant with the interposition of pauses. This phenomenon emphasizes the fact that the flowmeter measures the actual velocity of the medium presented to it under ambient conditions, and thus yields a faithful datum for consideration in the design of breathing equipment.

The breath patterns of a given individual look as unique as fingerprints, as noted by Fleisch (1), Bretschger (3), Gukelberger (4, 5) and others. A method of characterizing individual patterns quantitatively is not yet available, but may be feasible with a detailed statistical analysis of individual features of the breath pattern such as made in this series of tests.

From tables 1 and 2 it is seen that, with full oxygenation and submaximal dead space, the distribution of the data on the breathing pattern is probably influenced by the interaction of a group of factors, only a few of which were controlled in these tests, and many of which may well be individual variables. No attempt has been made here to sort the individual patterns into characteristic groups, although this is contemplated on a larger group of subjects.

The data indicate that the effect of altitude on the breathing mechanism is greatest on the expiratory phase, although it affects nearly all features of the act. Fleisch (2) reported, in this connection, that hypoxic subjects at altitude (up to 23,000 feet) introduced post-expiratory pauses despite the hyperventilation incurred by breathing ambient air. These did not occur in the illustration given by Pappenheimer and Lilly for 44,000 feet (9). It appears that ventilation in a rarefied atmosphere at adequate oxygen pressure is carried out so expeditiously via the proprioceptor controlled mechanisms that the chemical initiation of inspiration by CO_2 , which is governed normally by metabolic conditions, appears to lag more than at ground level. This deduction leads to the hypothesis that individuals vary in their ability to supply their bodies with oxygen and remove carbon dioxide via

the lungs, and that this is correlated with the degree to which they use interphasic pauses in their breath cycle. It appears possible to identify individuals with barely adequate ventilatory capacities by reducing or increasing the density of the respired gases, by simulating altitude, diluting with helium or krypton and noting the density level at which interphasic pauses appear. Substantiation of the effect of density on breath velocity by use of helium-oxygen mixtures has been carried out on a different group of volunteers.

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A COMPARISON OF THE RESPIRATORY ACTIVITY AND HISTOLOGICAL CHANGES IN ISOLATED PANCREATIC TISSUE

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DEUTSCH AND RAPER (1) found that the rate of respiration of isolated pancreatic tissue was increased by secretin and by acetyl choline with eserine. Harper and Mackay (2) studied the effects of secretin, pancreozymin and vagal stimulation upon the zymogen granule content of the cat's pancreas and the enzyme output in the pancreatic juice. They found that secretin administration, which resulted in the secretion of a juice of low enzyme content, did not affect the granule content of the cells. Vagal stimulation, on the other hand, or the administration of pancreozymin each resulted in an increased output of enzymes in the juice and a diminution in the granules in the cells.

The crude preparations of secretin used by Deutsch and Raper in 1936 probably contained pancreozymin. The present observations were made to compare the effects of pancreozymin and of pancreozymin-free secretin with those of acetyl choline and eserine on the respiration of isolated pancreatic tissue, and to correlate the respiratory effects of these agents with their effects on the zymogen granule content of the isolated tissue. In addition a few observations were made on the effects of histamine and gastrin on isolated pancreatic tissue.

In order to determine whether the energy required for secretion of zymogen granules could be supplied by anaerobic reactions, experiments were carried out in the absence of O_2 , but no secretion was observed even when adenosine triphosphate was added (Cf. Lipmann, 3).

METHODS

The experiments were performed on isolated pieces of cat pancreas. The animals were fasted overnight. In early experiments the cat was anesthetized with ether followed by chloralose. After an hour or two the pancreas was removed, care being taken to avoid touching the tissue with the fingers, which might possibly initiate secretion by contamination with acetyl choline from the skin. In later experiments the animal was killed by a blow on the head and the pancreas was then removed and used at once.

Samples of the tissue were freed from adherent matter, rapidly weighed on a torsion balance, minced with sharp, small and fine-pointed scissors and placed in the main compartment of a Warburg cup. The pieces of minced tissue were small enough to ensure adequate oxygenation *in vitro* (4). The O_2 uptake, the resultant

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of O_2 uptake and CO_2 output, and the anaerobic glycolysis were measured with Warburg manometers in the usual ways at $38^\circ C$. (4). Conical cups (20-30 ml. capacity), with a center well and either one or two side arms were used. Anaerobiosis was maintained with a small stick of phosphorus, and CO_2 was absorbed by 0.2 ml. 2N-NaOH in the center well. The cups were equilibrated for 20 minutes in the bath, and thereafter measurements of the gaseous exchanges were made every 5 minutes. When the resting rate of respiration had been obtained (usually within 10 min. of equilibration), acetyl choline or other agents were added from the side arms. Readings were continued until the tissue had been in the bath for 60 minutes. The values of the Q_{O_2} and $Q_{CO_2}^{N_2}$ were expressed in $\mu l/mg.$ dry wt/hr.

The physiological salt solution (bicarbonate saline) of Krebs and Henseleit (5), gassed with 5 per cent CO_2 + 95 per cent O_2 or 5 per cent CO_2 + 95 per cent N_2 , was used during measurements of the balance of the O_2 uptake and the CO_2 output, or of the anaerobic glycolysis of cat pancreas, and the phosphate saline of Krebs (6) during experiments in 100 per cent O_2 . The glucose concentration was 0.2 per cent.

For the estimation of the initial dry weight of tissues, control samples of the pancreas were weighed, washed in distilled water and dried overnight at $110^\circ C$. This gave the ratio wet wt/dry wt. for the tissue, from which the (initial) dry weight of the samples incubated could be calculated.

Histological observations were made on the pieces of tissue which had been used for the respiratory experiments, and on pieces which had either been placed in the fixative solution immediately after removal from the animal, or had been placed in saline solution in the Warburg apparatus, but not stimulated in any way. The tissues were fixed and stained by Bensley's Neutral Gentian method as described by Harper and Mackay (2).

The histological observations were concerned with the zymogen granule content of the cells. This is expressed in the tables below as ++++ (cells about $\frac{3}{4}$ filled with granules); +++ (about $\frac{1}{2}$ filled with granules); ++ (about $\frac{1}{4}$ filled with granules); + (less than $\frac{1}{4}$ filled with granules). Where marked cell damage was observed no interpretation was attempted.

The secretin and pancreozymin were prepared by a modification of the method described by Harper and Raper (7). The secretin preparations were quite free of pancreozymin and the pancreozymin preparations showed only a trace of secretin activity. The gastrin was prepared by adsorption on a bile acid precipitate from a 60 per cent alcohol extract of the antral mucosa, as described by Harper (8). The material was a powerful stimulant of acid gastric secretion in experiments on cats. In most of these experiments it had no effect on the pancreas, but in a few it showed faint traces of pancreozymin activity.

RESULTS

Control observations of two types were made: a) Pieces of tissue were placed straight in the fixative so that the initial appearance of the gland could be observed. b) Pieces of tissue were incubated in saline alone, in order to determine the resting metabolism of the gland and to see whether under these conditions any diminution in

granule content occurred. It was found that the metabolism was constant or fell slightly. There was in no instance an increase above the initial rate. Slight diminution in zymogen granule content was observed in 4 out of 19 experiments. The resting Q_{O_2} in 69 samples of isolated pancreatic tissue from a total of 16 cats was from -2.0 to -9.8 , average -4.27 , standard deviation 1.97 .

Acetyl choline with eserine. The effects of these agents are illustrated in the results of an experiment in table 1. The effects observed were an increase in respiration with a concomitant decrease in zymogen granules. Acetyl choline, final concentration 0.01 mg. per cent with eserine, final concentration 0.01 mg. per cent had no effect on the Q_{O_2} (2 experiments). When the same drugs were used at a concentration of 0.1 mg. per cent, the Q_{O_2} was increased (112% – 193% ; average 140% , 8 experiments). An experiment at a concentration of 1.0 mg. per cent showed 126 per cent of the resting value. In one experiment with a concentration of 10 mg. per cent, an increase to 156 per cent was observed. Experiments in the bicarbonate saline showed that the increased uptake of O_2 following stimulation was concomitant

TABLE 1. EFFECTS OF ACETYL CHOLINE WITH ESERINE ON RESPIRATORY ACTIVITY AND HISTOLOGICAL APPEARANCE OF ISOLATED PANCREATIC TISSUE (1 EXPERIMENT)

TREATMENT OF TISSUE	MAXIMUM RESPIRATORY RESPONSE AS COMPARED WITH CONTROL IN SALINE.	ZYMOMEN GRANULE CONTENT OF THE CELLS
	$\frac{Q_{O_2} \text{ MAX.}}{Q_{O_2} \text{ CONTROL}} \times 100$	
Control sample straight in fixative.....		++++
Control sample in saline.....	100	++++
Acetyl choline 0.1 mg. % eserine 0.1 mg. %.....	136	+++
Acetyl choline 1.0 mg. % eserine 1.0 mg. %.....	126	+
Acetyl choline 10.0 mg. % eserine 10.0 mg. %	156	+++

with a similar increased output of CO_2 . There were no histological differences observed between tissue incubated in the bicarbonate or in the phosphate salines.

Effects of pancreozymin and secretin. The effects of pancreozymin are illustrated by the results in tables 2 and 3. There was, with the addition of pancreozymin, an increase in respiration. The histological changes observed following the addition of pancreozymin were similar to those following acetyl choline, i.e. a migration of the zymogen granules towards the acinar end of the cell took place and a diminution in the amount of granular material in the cell along with a filling of the ducts with stainable material (where previously they had been empty). Secretin promoted no reduction in the zymogen granule content of the cells but an apparent increase in the zymogen granule content was occasionally observed. This was due to a scattering of the zymogen granules in the cell.

Atropine. At concentrations of either 0.1 or 1.0 mg. per cent atropine completely prevented the increase in respiration following acetyl choline with eserine, but it had no effect on the increased rate of respiration following pancreozymin (6 experiments).

Histamine. Five experiments were carried out with histamine. In one in which 0.1 mg. per cent of histamine was added, no stimulation of respiration or alter-

TABLE 2. EFFECTS OF PANCREOZYMIN AND SECRETIN ON RESPIRATORY ACTIVITY AND HISTOLOGICAL APPEARANCE OF ISOLATED PANCREATIC TISSUE

TREATMENT OF TISSUE	EXPERIMENT A		EXPERIMENT B	
	$\frac{Q_{O_2} \text{ max.}}{Q_{O_2} \text{ control}} \times 100$	Zymogen content of the cells	$\frac{Q_{O_2} \text{ max.}}{Q_{O_2} \text{ control}} \times 100$	Zymogen content of the cells
Control straight in fixative.....		++		
Control in saline.....	100	++	100	++
Pancreozymin 3.0 mg. %.....			118	+
Pancreozymin 30.0 mg. %.....			143	+
Pancreozymin 3.0 mg. %.....	120	+		
Secretin 2.0 mg. %.....		+		
Pancreozymin 30.0 mg. %.....	134	+		
Secretin 2.0 mg. %.....				
Secretin 0.2 mg. %.....	143	++	136	+++ (scattering of granules)
Secretin 2.0 mg. %.....	190	++		

TABLE 3. EFFECTS OF PANCREOZYMIN AND SECRETIN ON RESPIRATORY ACTIVITY OF ISOLATED PANCREATIC TISSUE

TREATMENT OF TISSUE	$\frac{Q_{O_2} \text{ MAX.}}{Q_{O_2} \text{ CONTROL}} \times 100$		
Pancreozymin 3.0 mg. %	153	118	153
Pancreozymin 10.0 mg. %	116		
Pancreozymin 30.0 mg. %	147 114	143	148
Pancreozymin 3.0 mg. %, Secretin 0.2 mg. %	120	136	136
Pancreozymin 30.0 mg. %, Secretin 2.0 mg. %	134	142	144
Secretin 0.2 mg. %	143 109 112	136 111 106	120 109
Secretin 2.0 mg. %	190 111	110	133

ation in the histological appearance of the cells was observed. In the remaining 4 experiments where 1.0 mg. per cent of histamine was employed, there was no effect in one and a slight increase in respiration in the other 3 (104, 105, 130; control = 100). In one experiment alone, slight diminution in zymogen granules was observed. Gastrin. Four experiments were carried out with concentrations of gastrin

of 0.6, 6.0 and 12.0 mg. per cent. Small increases in the rate of respiration were observed at the higher concentrations in 2 experiments (116 and 106; control = 100). At the highest concentration a slight decrease in zymogen granules was observed.

Experiments in the absence of oxygen. In 11 experiments the anaerobic glycolysis was from 3.0 to 4.5 (average 3.7, standard deviation 0.52). In such experiments the rate of glycolysis and the histological appearance of the cells was unaffected by the addition of acetyl choline with eserine, either alone or with ATP in (final) concentrations varying from 0.00001M to 0.01M.

DISCUSSION

The observations of Deutsch and Raper (1) that acetyl choline and secretin increase the rate of respiration of isolated cat pancreas have been confirmed. These experiments show that isolated pancreatic tissue can also be used to study the histological changes caused by a variety of agents. The results show that pancreozymin and pancreozymin-free secretin act as respiratory stimulants: the zymogen granules in the cells were decreased by the addition of pancreozymin or of acetyl choline with eserine but not by secretin. The slight stimulant effect of gastrin on the respiration of pancreatic tissue may be due to traces of pancreozymin in the preparation. Atropine does not prevent the increase in respiration following the addition of pancreozymin but completely inhibits both the respiratory and histological changes due to acetyl choline. These observations confirm those of Harper and Mackay (2).

Experiments showed that secretion does not occur in the absence of oxygen. The fact that the addition of ATP to the anaerobic tissue failed to cause secretion does not prove that ATP plays no part in the process since no evidence is available that this compound entered the pancreatic cells.

SUMMARY

A correlation has been made of the effects on respiration and on the histological appearance of cat pancreatic tissue stimulated *in vitro* by secretin, pancreozymin and acetyl choline with eserine. Secretion of zymogen granules was initiated and respiration stimulated by acetyl choline with eserine and by pancreozymin. Atropine inhibits the actions of acetyl choline but not those of pancreozymin. Secretin stimulates respiration but does not promote secretion of zymogen granules.

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COMPARISON OF RESPIRATION AND GLYCOLYSIS IN THE BRAINS OF NORMAL AND FEBRILE RABBITS¹

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THE maintenance of the body temperature of homoiotherms at a fairly constant level by operation of the hypothalamic temperature regulating centers implies that some temperature-sensitive process within the neurons of the centers must be so organized as to yield the equivalent of the set of a thermostat. The nature of this process is not known. The hypothesis that the rate of a key temperature-sensitive intracellular process is a factor determining this set level is under investigation in this laboratory.

The rise in body temperature following the administration of pyrogenic agents is commonly attributed to an elevation of this set level. According to the hypothesis stated above, the rate of the key metabolic process should be altered in the brain tissue of febrile animals. Since the majority of the energy-yielding processes now recognized influence measurements of oxygen consumption, glycolysis or both, these metabolic processes have been chosen in our search for a possible key reaction. Technical difficulties precluded direct investigation of the hypothalamic tissue. However, because of the qualitative similarity of the metabolic pattern in the several parts of the central nervous system (1), quantitative changes appearing in one region might well be paralleled by changes in a similar direction in other regions. Accordingly we have investigated the rates of respiration and of anaerobic glycolysis in cerebral cortex slices from normal rabbits and from rabbits with fever produced by the administration of a bacterial pyrogen. The results are presented in this paper.

METHODS

Adult white rabbits weighing from 1.8 to 2.7 kg. were used. The animals were killed by injection of air into the marginal ear veins. The brain was rapidly removed and cerebral cortex slices were prepared by the cold moist box technique which has been described previously (2, 3). This procedure keeps the tissue in a cold moist environment from the time of excision until the respirometers are placed in the constant temperature bath. Thus in studies on oxygen consumption, imbalance between the aerobic and anaerobic phases of metabolism is minimized during the period of tissue manipulation (2, 4, 5), and in anaerobic work the overall metabolism is kept at a low level until suitable conditions are provided for the supply of nutrients and the removal of metabolic end-products (2). The wet weight of most tissue sam-

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ples was 40 to 60 mg. Control experiments have shown that under the conditions of these experiments oxygen consumption is proportional to the initial wet weight of tissue over the range 10 to 90 mg. Slice thickness was 0.4 to 0.5 mm. (cf. 2). Immediately after weighing, tissue samples were placed in respirometer flasks or in small weighing bottles. The latter group of samples was dried to constant weight in an electric oven at 105°C.

Respiration was measured by the Warburg manometric method (2, 6, 7). The gas phase was oxygen. The liquid phase was Krebs' Ringer's phosphate (8) containing 0.011 M glucose. The center wells of the flasks contained 5 per cent KOH with Whatman no. 40 filter paper wicks. Manometric measurements were made in a constant temperature bath at $39.0 \pm 0.01^\circ\text{C}$. after 15 minutes of thermoequilibration. Readings were taken at 10-minute intervals for a minimum of 60 minutes. Oxygen consumption was constant during this time. Results are expressed in the conventional 'Q' notation (cf. 2). Thus Q_{O_2} denotes microliters of oxygen consumed, measured under standard conditions, per mg. initial dry weight of tissue per hour.

Anaerobic glycolysis was measured by the manometric method (6, 7). The gas phase was 95 per cent N_2 -5 per cent CO_2 which had been passed through a Savage-Ordal (9) hot reduced copper tube to remove traces of oxygen. Uniform gassing was effected by passing this mixture through the respirometers in series. After leaving the last respirometer the gas was passed through a Wolff bottle so that the rate of flow could be observed and maintained. Gassing was carried out for a minimum of 10 minutes after the respirometers had been placed in the constant temperature bath. The liquid phase was Krebs-Henseleit (10) bicarbonate solution. This medium had previously been gassed for over an hour with the same oxygen-free mixture used as the gas phase in the respirometers. Manometric measurements were made in a constant temperature bath at $39.0 \pm 0.01^\circ\text{C}$. Readings were taken at 10-minute intervals for 40 to 60 minutes. The rate of glycolysis was constant during this time. Results are expressed in the conventional 'Q' notation. Thus $Q_A^{N_2}$ denotes microliters of acid produced, measured as a gas under standard conditions, per mg. initial dry weight of tissue per hour. All Q values given in tables were calculated from measurements made during the period of steady state.

Fever was induced in those animals referred to as 'febrile' by injection of 0.05 ml. of sterile typhoid-paratyphoid triple vaccine² (hereinafter termed T.P.T.) into the marginal ear vein. Rectal temperature was followed (taking care not to disturb the animals, cf. 11) for an hour. No animal was used unless rectal temperature rose at least 1°C . in this time. All febrile animals were killed exactly one hour after injection of T.P.T.

RESULTS

Table 1 shows the results of measurements of oxygen consumption in normal and in febrile rabbit cerebral cortex slices at 39°C . The mean values of Q_{O_2} are 8.76 and 8.58 respectively. The significance of the observed difference between these

² This vaccine, prepared by the Cutter Laboratories, Berkeley, California, contained in each ml. 1000 million *E. typhosa*, 500 million *S. paratyphi* and 500 million *S. schottmuelleri*. The preservative was 0.25 per cent tricresol.

two means was evaluated by the use of Student's 't'-test (cf. Snedecor, 12). The values of *t* and *P* obtained were 0.659 and approximately 0.5 respectively. Thus there appears to be no significant difference in respect of rate of oxygen consumption at 39°C. between cerebral cortex slices from normal and from febrile rabbits.

The results of a similar comparison between the rates of anaerobic glycolysis in cerebral cortex slices from normal and febrile rabbits at 39°C. are shown in table 2. Here again it was found by application of Student's 't'-test that the observed difference between the means was not significant.

Comparison of the variances (squared standard deviations) by the "F" or variance ratio method (cf. 12) indicates that there is no significant difference in the variability of Q_{O₂} and of Q_A^{N₂} between the normal and febrile rabbits.

TABLE 1. COMPARISON OF OXYGEN CONSUMPTION AT 39°C. OF CEREBRAL CORTEX SLICES FROM NORMAL AND FEBRILE RABBITS (FOUR ANIMALS (12 TISSUE SAMPLES) IN EACH GROUP)

	NORMAL	FEBRILE
Mean Q _{O₂}	8.76	8.58
Range: Q _{O₂}	7.83-10.15	7.67-9.63
Standard deviation.....	0.690	0.646
No. of observations.....	12	12

Comparison of means: *t* = 0.659; *P* 0.5.

TABLE 2. COMPARISON OF ANAEROBIC GLYCOLYSIS AT 39°C. OF CEREBRAL CORTEX SLICES FROM NORMAL AND FEBRILE RABBITS (SIX ANIMALS (48 TISSUE SAMPLES) IN EACH GROUP)

	NORMAL	FEBRILE
Mean Q _A ^{N₂}	12.58	12.27
Range: Q _A ^{N₂}	8.49-21.13	7.88-19.53
Standard deviation.....	2.590	2.561
No. of observations.....	48	48

Comparison of means: *t* = 0.590; *P* 0.5.

DISCUSSION

These results show that the altered thermostatic behavior by which a bacterial pyrogen appears to induce fever in the rabbit is not accompanied by alteration of either the oxygen consumption or anaerobic glycolysis rates, at 39°C., of cerebral cortex tissue removed one hour after the injection of the pyrogen. Thus these findings lend no support to the concept presented in the introduction to this paper that the cellular metabolism of the central nervous system is a factor determining the level at which the thermoregulatory processes operate. However they do not necessarily invalidate this hypothesis for the following reasons: *a*) The pyrogen may influence thermostatic behavior by affecting processes other than those determining the rates of oxygen consumption or anaerobic glycolysis. *b*) The assumption of qualitative similarity of metabolic pattern in the several parts of the central nervous system may be incorrect, the pyrogen affecting hypothalamic but not cortical tissue metabolism. *c*) The pyrogen might produce metabolic effects *in vivo* which are lost when tissue slices are studied *in vitro*. *d*) A possible metabolic effect of the pyrogen may have appeared and waned by the end of the first hour. Grant (13) has shown that the vasoconstriction and suppression of thermal polypnea (which

are the principal causes for the febrile temperature rise in the rabbit) are replaced by vasodilatation and polypnea at approximately one hour after injection of T.P.T., the time at which the animals were killed for the metabolic studies reported here. However this objection is weakened by the finding that some 30 minutes later vasoconstriction and polypnea suppression return without further pyrogen administration. Further, during the period of vasodilatation and polypnea, the respiratory rate may not be as high as it was before injection in spite of the presence of a two degree rise in rectal temperature, which suggests that the factor (presumably the pyrogen) depressing the respiration is still active.

In view of these considerations the possibility that thermoregulatory behavior may be related to the metabolic activity of the central nervous system remains an open question.

SUMMARY

The mean Q_{O_2} values at 39°C. for cerebral cortex slices from normal rabbits and from rabbits with fever produced by administration of typhoid-typhoidpara vaccine were 8.76 and 8.58 respectively. The difference between these means is not statistically significant. The mean $Q_A^{N_2}$ values at 39°C. for cerebral cortex slices from normal and febrile rabbits were 12.58 and 12.27 respectively. The difference between these means is not statistically significant.

An hypothesis relating the level at which body temperature is regulated to the metabolic activity of the central nervous system is stated and the bearing of these findings on that hypothesis is discussed.

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INFLUENCE OF ANTIPYRINE ON RESPIRATION, GLYCOLYSIS AND CHOLINESTERASE ACTIVITY IN RAT BRAIN¹

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IN THE introduction to the preceding paper in this volume an hypothesis relating thermostatic behavior of homoiotherms to central nervous system function was set forth. In this paper are reported experiments designed to subject this hypothesis to a further test by determining whether antipyrine, a substance reducing body temperature, affects certain metabolic activities of central nervous system tissue.

METHODS

Adult albino rats of the Slonaker-Wistar strain were used. The procedures employed in the preparation of cerebral cortex slices and for the measurement of respiration and glycolysis were the same as described previously (1) except that the animals were killed by decapitation rather than by intravenous injection of air as in the case of the rabbits. When oxygen consumption was measured the sidearms of the respirometer flasks contained graded amounts of antipyrine (Merck) made up in the same Krebs-Ringer's phosphate glucose solution (2) used as the suspension medium. The contents of the flasks were added to the main compartments of the flasks after a 30-minute control period (which followed 15 minutes of thermoequilibration), so that control and 'antipyrine added' runs were made with the contents of each flask. This procedure was not followed in the measurement of glycolysis because the handling necessary to make additions from the vessel sidearms disturbed the otherwise even rate of carbon dioxide evolution. In order to avoid this effect the desired concentrations of antipyrine were made up in the Krebs-Henseleit bicarbonate glucose medium (3) used in the main compartments of the flasks. Thus there was no 'pre-addition' control period as in the case of the respiration measurements and comparisons were made against controls containing no antipyrine in the liquid phase.

Cholinesterase activity was measured manometrically (4-6). The general procedure has been described previously (6). The enzyme extract used was the 'supernatant fraction' of Nachmansohn and Feld (7). This was prepared from whole brain homogenized in cold (0°C.) calcium-free Krebs-Henseleit bicarbonate solution, together with the desired amounts of antipyrine. The sidearms contained acetylcholine chloride (Merck) made up in the same solution. The contents of the side-

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arms were added to the main compartments of the flasks at the end of thermoequilibration. The final concentration of acetylcholine was 0.015 M. The gassing procedure was the same as in the experiments on anaerobic glycolysis (1). The pH of the liquid phase in the flasks, in equilibrium with the gas mixture 95 per cent N_2 -5 per cent CO_2 , was 7.4.

All of the manometric measurements were made in a constant temperature bath at $37.5^\circ \pm 0.01^\circ C$. When oxygen was the gas phase the respirometers were gassed before being placed in the water bath. When the 95 per cent N_2 -5 per cent CO_2 mixture was used gassing was accomplished during the 15 minute thermoequilibration period which preceded all runs.

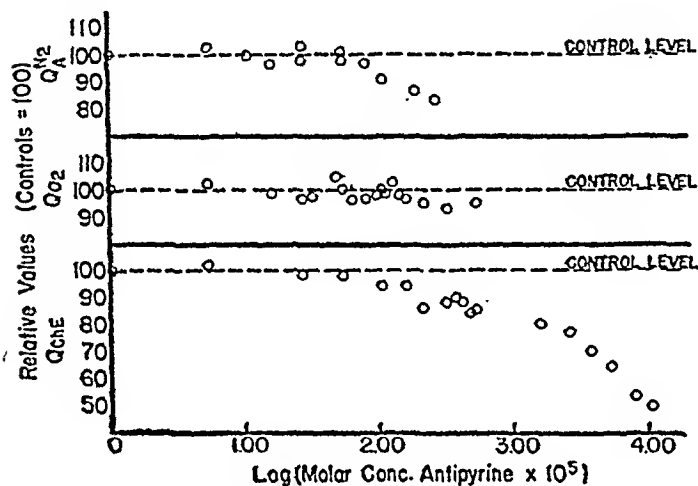


Fig. 1. GRAPH SHOWING EFFECT OF GRADED CONCENTRATIONS of antipyrine on oxygen consumption and anaerobic glycolysis in rat cerebral cortex slices and on cholinesterase activity in the supernatant fraction of whole rat brain homogenate at $37.5^\circ C$.

Respiration and glycolysis data are expressed in the conventional 'Q' notation on the initial dry weight basis (cf. 1). In all cases the oxygen consumption was constant for several hours and glycolysis for 40 to 60 minutes. Values given in the figure and table were calculated from readings made during steady state periods. Cholinesterase activity is expressed in terms of milligrams of acetylcholine hydrolyzed in one hour by 1.0 ml. of supernatant fraction from an homogenate containing 100 mg. of fresh whole rat brain per ml. The symbol Q_{chE} is used to denote this (cf. 8). Readings were taken at 5-minute intervals for a period of 40 minutes. The rate of CO_2 evolution was constant during this time.

RESULTS

Anaerobic glycolysis. Figure 1 shows the effect of antipyrine on the rate of anaerobic glycolysis in rat cerebral cortex slices. Concentrations ranging up to about $5.3 \times 10^{-4} M$ had no effect. With further rise in antipyrine concentration an inhibition was observed which amounted to 17 per cent at the highest concentration used ($2.6 \times 10^{-3} M$). There is no indication of an augmentation phase of anaerobic glycolysis caused by antipyrine such as has been observed when the effect of graded concentrations of another antipyretic, magnesium chloride, was investigated (10).

Respiration. It is shown in figure 1 that the respiration of rat cerebral cortex slices was not affected by antipyrine up to a concentration of about $2 \times 10^{-3} M$. With further increase in antipyrine concentration a slight inhibition of oxygen con-

sumption was observed. However this amounted to only 5 to 7 per cent of the highest concentration of the drug tested ($5.3 \times 10^{-3}M$). Thus the respiratory process in rat cerebral cortex resembles that of rat liver suspensions (9) in respect of stability toward antipyrine.

Cholinesterase activity. It is shown in figure 1 that antipyrine, in concentrations up to about $5.3 \times 10^{-4}M$ has no effect on the cholinesterase activity of the supernatant fraction of rat brain homogenate. With increasing concentrations of the drug inhibition develops. Fifty per cent inhibition was observed at the highest concentration studied, $10.6 \times 10^{-2}M$. Thus our rat brain cholinesterase preparation was considerably less sensitive toward antipyrine than human serum cholinesterase, with which 50 per cent inhibition occurred at a concentration of antipyrine less than one tenth as great (11, 12).

Control experiments: Statistics. A considerable number of control experiments were run in the course of this investigation. The data so obtained provide values of Q_{O_2} and $Q_A^{N_2}$ of rat cerebral cortex slices and of the cholinesterase activity of the

TABLE 1. MEANS AND OTHER STATISTICAL DATA OF CONTROL EXPERIMENTS ON RESPIRATION AND ANAEROBIC GLYCOLYSIS IN RAT CEREBRAL CORTEX SLICES AND CHOLINESTERASE ACTIVITY IN THE SUPERNATANT FRACTION OF WHOLE RAT BRAIN HOMOGENATE; $37.5^\circ C$.

	Q_{O_2}	$Q_A^{N_2}$	ACh HYDROLYZED/ HOUR/1.0 ML. SUPERNATANT
Mean.....	11.13	9.26	2.63
Range.....	9.85-12.71	5.04-12.76	2.40-2.89
Standard deviation.....	0.229	1.685	0.126
No. of runs.....	30	27	15

For description of units see text.

supernatant fraction of whole rat brain homogenate at $37.5^\circ C$. which may be of some use to other workers in the field of tissue metabolism. The absolute values of the mean rates of these processes together with certain derived statistics are given in table 1.

DISCUSSION

The bearing of these results on the hypothesis concerning the mechanism of temperature regulation formulated in the introduction to this paper may now be considered. The intravenous dose of antipyrine which in the rabbit causes increased respiration, dilatation of ear vessels and a fall in rectal temperature ranges from 0.06 to 0.25 gm/kg. body weight. Data concerning the resultant blood concentrations are not available, but on the assumption that antipyrine is uniformly distributed throughout the body these doses would yield concentrations from $3.2 \times 10^{-4}M$ to $1.3 \times 10^{-3}M$. Such concentrations, according to the findings reported in this paper, are somewhat below the minimum required for depression of oxygen consumption *in vitro*. However, the concentrations estimated to result from the highest doses are within the range in which some depression of anaerobic glycolysis and cholinesterase activity of brain tissue might occur.

It may be noted that magnesium chloride, which also causes increased respiration, dilatation of ear vessels and lowered body temperature (13), has an effect on the rate of anaerobic glycolysis in cerebral cortex tissue in the direction opposite to that of antipyrine (10). Magnesium chloride, in the concentration range concerned, resembles antipyrine in leaving the rate of oxygen consumption unaffected.

As far as conclusion from a study of the two agents, antipyrine and magnesium, may be warranted, it would appear that activation of heat defense mechanisms by a drug bears no constant relation to its influence on anaerobic glycolysis in nervous tissue and can occur in the absence of any change in oxygen consumption.

While it is obvious that these considerations lend no support to the hypothesis under discussion (see introduction), the limitations on the applicability of this type of metabolic data to the evaluation of that hypothesis, which have been set forth in the preceding paper, are such that we consider the hypothesis to warrant further testing.

SUMMARY

Antipyrine, when added to the medium in which slices of rat cerebral cortex are suspended, has no effect on the rate of oxygen consumption at 37.5°C. in concentrations below $2 \times 10^{-3}M$. At concentrations greater than this, slight depression of respiration occurs. Anaerobic glycolysis in brain slices and cholinesterase activity in brain homogenates are depressed by concentrations of antipyrine above $1 \times 10^{-3}M$. The question of the possible bearing of these metabolic effects of antipyrine on its antipyretic action is discussed.

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ACTION OF METHYL FLUOROACETATE ON RESPIRATION AND POTENTIAL OF NERVE¹

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THERE is general agreement that pyruvate is a key link in carbohydrate oxidation and glycolysis. Whether the further oxidation of pyruvate is via a simple decarboxylation or through the Krebs cycle is not known for nerve. Although glucose is burned and fermented by resting nerve, the excess metabolism of activity may involve phospholipid breakdown (1). Energy from these reactions serves to build energy-rich phosphate bonds, stored in nerve mostly as CrP (2), which probably are the more immediate energy source for maintaining cell integrity and function. The fluoroacetates are known to interfere with pyruvate oxidation, and perhaps with that of acetate separately (3), to interfere in the Krebs cycle, and to block specifically the extra oxygen consumption of activity in the case of muscle (4); as does azide in muscle (5) and nerve (6, 7). Correlated studies on the influence of this inhibitor on the respiration and functional properties of nerve should thus yield information as to the metabolic basis of nerve activity.

METHODS

Sciatic nerves of summer, winter and spring green frogs, with perineurium slit to facilitate diffusion, were mounted singly in a three-compartmented chamber. Electrodes of Ag-AgCl or of Pt were arranged as follows: in an end compartment, two stimulating electrodes; in the middle one, two pick-up electrodes (leads A and B); in the far end one, a pick-up (lead C) and an indifferent electrode. Dimensions are shown in figure 5. The central compartment was kept filled with Ringer's or a drug solution, substitution being made without moving the preparation. Stimulation, normally only during measurements, was at 20 per second by a conventional thyatron stimulator with a transformer output. Action potentials were measured on the cathode ray tube face for height or photographed or traced for study of shape. Experiments were begun within half an hour of killing the frog and spike heights normally remained constant for over 6 hours. A 30- to 60-minute control period always preceded the application of test solutions. Electrical studies were made at room temperature, 21° to 28°C. in various experiments.

Respiration studies were carried out in the microrespirometer previously described (8) or, in stimulation experiments, in a new modification (7). For resting QO_2 , 3-mg. stretches of nerve were run at 30°C. (summer, 1947); for active QO_2 , 10-mg. stretches (summer, 1948). Solutions were made in Ringer-bicarbonate, reagent being substituted for a molar equivalent of NaCl when any serious departure from isotonicity was involved, and adjusted to pH 6.8-7.4. The MFA was tested for purity by boiling point determination.³

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² Predoctoral Public Health Fellow.

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RESULTS

Sodium fluoroacetate is almost inert for frog nerve. Spike height was unaffected at concentrations up to 100 mM, with the nerve left mainly at rest or, compared to a control, when tetanized continuously for 13 hours. Even at pH 6.0, which should

TABLE I

INHIBITOR	% INHIBITION OF RESPIRATION		
	2 hrs.	3 hrs.	4 hrs.
<i>Sodium fluoroacetate</i>			
0.01M.....	-8 (6) ¹	3	7
0.10M.....	(3)	40	55
<i>Methyl fluoroacetate</i>			
0.005M.....	80 (6)	80	80
0.001M.....	25 (3)	30	35

¹ Number of experiments indicated in ().

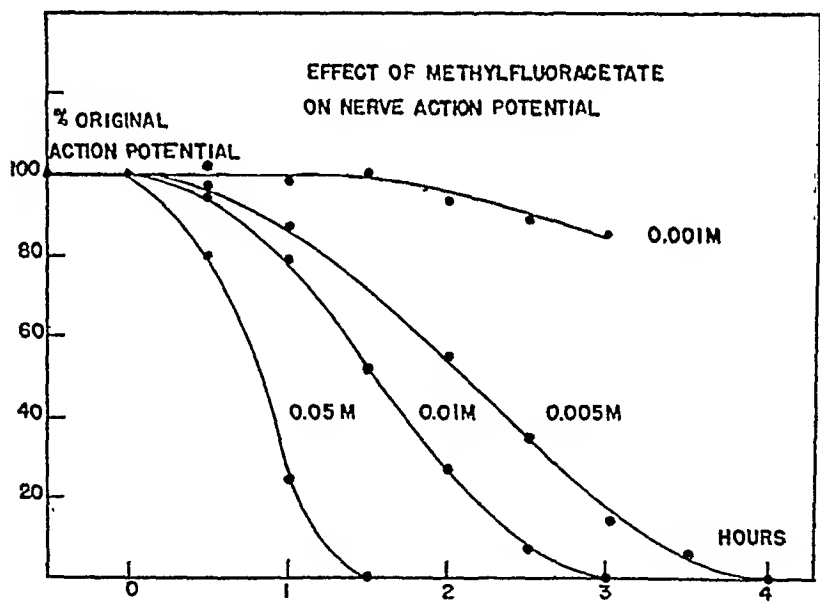


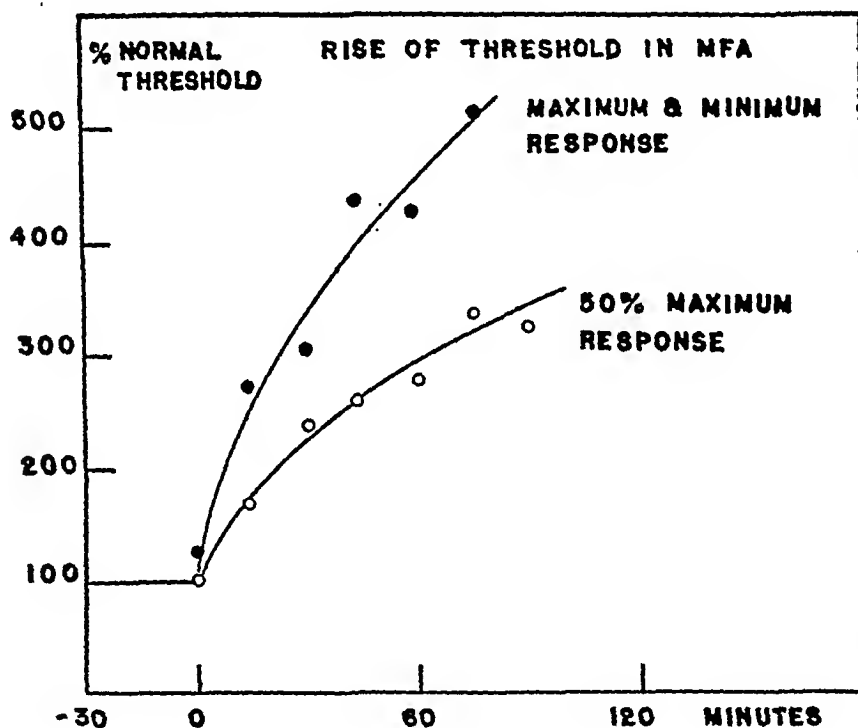
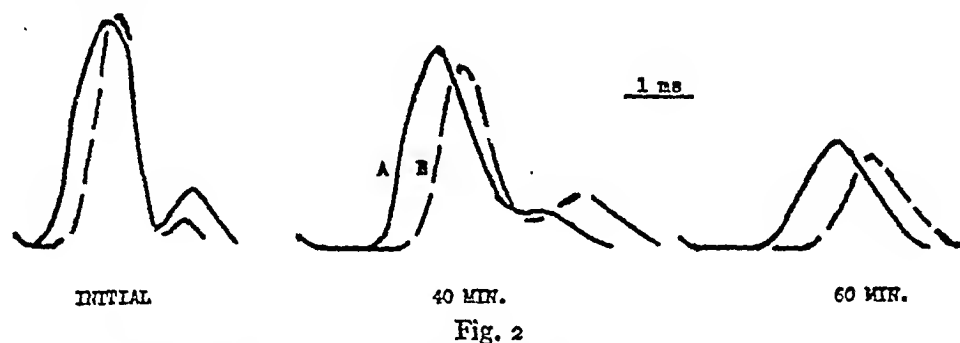
Fig. 1

favor penetration by un-ionized molecules, no action potential changes were produced.

Respiration of paired nerves agreed within 10 per cent (Q_{O_2} at 30°C. = 142 ± 29 in 36 experiments), and no change resulted from soaking as long as 4 hours in 10 mM Na fluoroacetate (table I). At 100 mM, oxygen consumption was reduced 50 per cent, the spike remaining normal. The feeble action of this substance is, therefore, not entirely due to lack of penetration; a conclusion supported by its similar lack of activity in inhibiting dehydrogenases in nerve or brain homogenate (9).

Methyl fluoroacetate (MFA), in contrast, blocks nerve in concentrations lower than does DFP (10, 11), though not so low as cyanide (12) or IAA (13). One mM

solutions are ineffective, but at 5 mM block is complete in 3 to 4 hours and at 50 mM, within 1.5 hours (fig. 1). Continued tetanization does not alter the curve of fall, in contrast to the IAA-poisoned nerve (13). Spike height falls along a sigmoid curve, as if a latent period exists in the action of MFA (but see thresholds, below), longer with lower concentration, but the shape of the curve is more directly determined by the order of failure of individuals in the fiber population. Thus, spike height at



the C electrode, beyond the exposed stretch of nerve, falls with or slightly ahead of that at the A electrode on the exposed region. Further, at the B electrode, more distal in the middle compartment, the potential falls with that at C, while at A, more proximal, the fall is less rapid. A particular fiber, then, may block anywhere along its exposed length but its spike remains essentially at full value up to the point and time of block. Changes in shape of the nerve action potential (fig. 2) show a decrease of conduction velocity paralleling a rise in threshold before fibers begin to block. Block then develops progressively from the larger to the smaller fibers, without further slowing. Threshold, for minimal, 50 per cent, or maximal spike response, begins to rise at once and increases 2- to 5-fold over 30 to 90 minutes, alike in either 0.005 or 0.01 M MFA (figure 3). No change (or a slight increase in some

cases) in demarcation potential, measured at the nerve end in MFA (.01 or .005 M) compared to an end in saturated KCl, was observed up to complete block.

Winter frog nerves are only about half as susceptible to MFA as are summer nerves. Five mM blocks in 5.5 to 6 hours in winter, as compared to 3 to 4 hours in summer, and the 'latent period' is about 2 hours. At 10 mM concentration, the fall begins, in winter, in 1 to 1.5 hours and block is complete in 2.5 to 3 hours.

Once spike height has begun to fall, washing in Ringer does not alter its course. The inhibition is, therefore, irreversible after exposure for an hour or so. This is different from the inhibition of CrP resynthesis in muscle, which is reversible on washing (4). In yeast a partial reversal of the inhibited respiration is obtained with acetate (14), and in *chilomonas* with pyruvate and alcohol (15). We have tested a number of substrates for their ability to prevent or reverse MFA inhibition of nerve.

The oxygen consumption of nerve is more sensitive to MFA than is conduction. Even 1 mM cuts respiration by 30 per cent, with no block, and 5 mM reduces respiration to 20 per cent of normal at a time when only half the fibers are blocked (table 2). This re-emphasizes the factor of safety (16) in the energy supply of nerve, seen also

TABLE 2

HRS. AFTER MFA (0.005M)	% OF NORMAL FOR FROG SCIATIC			
	No addition		Na Fumarate (0.05M)	
	Action potential	Respiration	Action potential	Respiration
1	85 (5)	(6)	100 (5)	(3)
2	50	20	100	45
3	15	20	100	55
4	0	20	100	

under conditions of hypoxia. The increased oxygen consumption on tetanization may be as much in nerves poisoned with MFA (1 to 5 mM) as in normal controls at times when the resting respiration of the MFA nerves has been cut as low as half normal. (Details will be published elsewhere, 7.) Addition of Na fumarate to normal nerve, up to 100 mM, has no effect on oxygen consumption; but added an hour before MFA, at ten-fold the molarity of the inhibitor, it reduces the inhibition from 80 per cent to only 50 per cent (table 2, fig. 4). With this increment in metabolism comes complete prevention of block.

In action potential experiments, each substrate was added an hour before the MFA (in similar substrate solution) and was tested in concentrations of 2 and 5 times that of the inhibitor (5 mM in summer, 10 mM in winter). Glucose, ethyl alcohol, acetate, pyruvate and alpha ketoglutarate were completely ineffective against MFA. Malate and perhaps oxaloacetate gave partial protection in 5:1 concentration, succinate protected fully at 5:1, partially at 2:1 (fig. 5). Most striking is the action of fumarate, which gave full protection for over 6 hours at twice the molarity of MFA (fig. 5.) This protection by fumarate can still be attained when it is added within 15 minutes after MFA, while after 30 minutes it is completely ineffective. This is further striking evidence of a brief 'latent period' in MFA action during which

its effects can be reversed, and of a succeeding period when damage has become irreversible even though conduction has hardly begun to fail. (That MFA action is not delayed by slow penetration is shown by the prompt increase in threshold.)

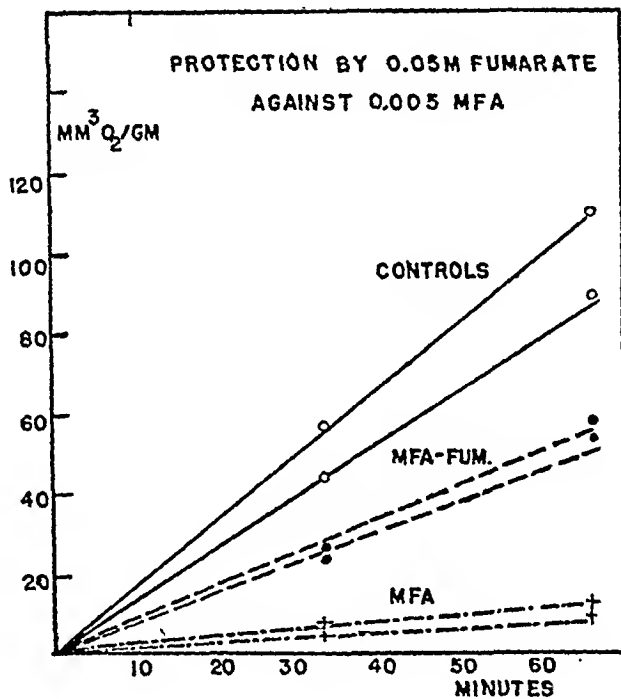


Fig. 4

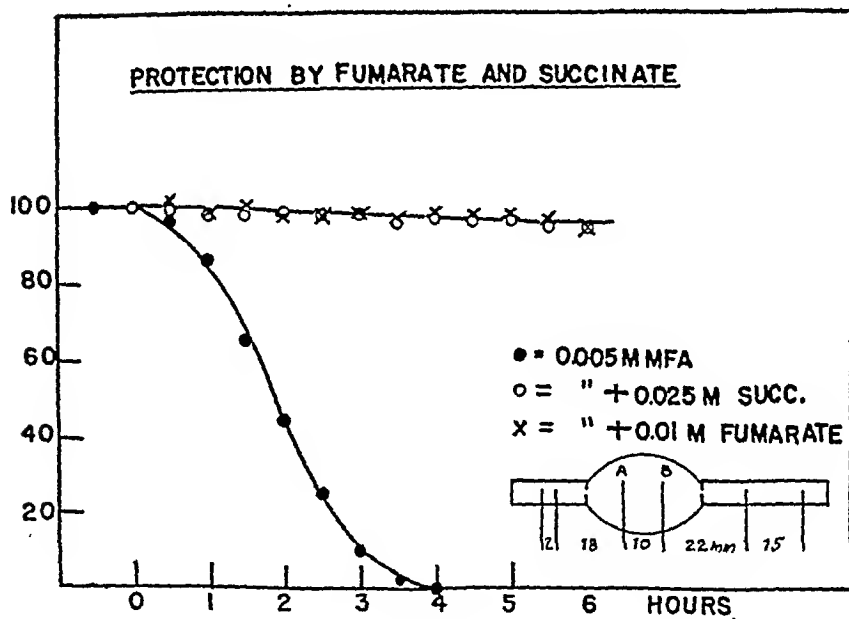


Fig. 5

This also speaks against MFA acting as a competitive inhibitor; although, on the other side, 20 mM fumarate protected against 10 mM MFA and 40 mM against 20 mM, but 20 mM fumarate did not protect against 20 mM MFA.

Controls with substrates on normal nerve showed them inactive—spike height

remained normal in 10 mM Na acetate for over 6 hours—except for malate and glucose added to Ringer in high concentration. The former, 100 mM, blocked conduction in two hours and even at 50 mM caused spike height to fall to half within 30 minutes, where it remained. Glucose, 100 mM, led to an increase in spike height of 15 to 20 per cent, which was maintained for hours. An increase followed by block, reported by others (17, 18) was probably due to fuller loss of electrolyte.

DISCUSSION

A nerve blocks, presumably, when the eddy currents between active region and to-be-activated region do not reach the threshold of the latter. This results, for unchanged geometry and conducting media, when the action spike is sufficiently decreased or the membrane threshold is sufficiently raised. While, in general, membrane potential decreases along with spike height, the two can vary independently. Similarly, membrane threshold and potential do not necessarily vary in parallel fashion. MFA blocks conduction by raising threshold (and slowing conduction), without altering the demarcation potential or the action spike. This resembles the narcotics (19, 20) or DFP (11), which also raise the nerve threshold; but not cyanide (19–21), anoxia (22), or IAA (23), which depolarize. With MFA, the threshold rise starts at once and is twice normal in 15 minutes, when conduction is slowed but otherwise unaltered. Even at 30 minutes, when the threshold is increased threefold, the action potential is over 95 per cent normal. A factor of safety of at least three is thus shown.

MFA does not decrease the metabolism of activity independently of that of rest. On the contrary, the extra oxygen consumption of conduction may be normal when the resting oxygen consumption has been cut below one-third, though it also decreases at higher MFA concentration before conduction fails. This is in sharp contrast to the action of azide, which can abolish the extra oxygen consumption of activity with disturbing resting respiration or conduction (6, 7), as well as to the action of MFA on muscle. Resting oxygen consumption, on the other hand, is depressed by MFA in concentrations and at times which still leave conduction intact. Even fumarate, which protects conduction fully against MFA block, leaves the resting oxygen consumption 50 per cent depressed by MFA. Clearly a factor of safety in the metabolic flow of energy exists, as earlier indicated (16). This result also suggests that MFA block of metabolism occurs above some energy-yielding steps, which continue to supply energy for function when oxygen uptake, nearer the top of the sequence, has been cut.

Metabolic energy, perhaps both resting and active together, is funneled into functional use, in nerve as in other tissues, through CrP and ATP (2). Interference with oxidations or glycolysis or both decreases the CrP content, but oxidative energy is the more important in maintaining normal conditions. Fluoroacetate acts as an inhibitor in the oxidative chain, just where is not fully agreed upon, but does not decrease lactic acid formation (4), and it especially prevents CrP re-synthesis and recovery oxygen consumption (4) and heat production (24) in muscle. (Metadinitrobenzene may decouple oxidations and phosphorylations, 23; and methylene blue, which, like MDB, markedly diminishes the creatin phosphate of muscle,

2, perhaps acts similarly. This action is not excluded for MFA.) Since the resting, but not the active, metabolism of nerve depends largely on carbohydrate oxidation (25), while the increase on activity is perhaps associated with phospholipid loss, it was thought that MFA, supposed at first to block acetate oxidation (3), might differentially depress the active metabolism in nerve even more than in muscle. This it does not do; and fuller evidence on MFA action (26, 3, 14) implicates pyruvate oxidation, presumably via the Krebs cycle (4). The sharp differences in the behavior of nerve and muscle—NaFA inactive on nerve (Dr. Stannard has, however, noted a similar inactivity on muscle), MFA action irreversible, and resting rather than active metabolism inhibited—emphasize again fundamental metabolic differences between these tissues.

That the Krebs cycle is operative in brain, is now well supported (27-29). In the case of nerve, the depressant action of arsenite (30), known to inhibit the oxidative decarboxylation of alpha ketoglutaric acid (31), suggests the cycle; while the failure of malonate, which inhibits the dehydrogenation of succinic acid, to depress resting potential (23) or spike, as we have found, speaks against it. The negative results cannot be dismissed simply as lack of penetration, since malonate is also relatively ineffective on the dehydrogenases of nerve brei (9). None the less, our findings that the tricarboxylic intermediates, especially fumarate, can prevent MFA block and counter its inhibition of respiration, speak for the presence and functional importance of a tricarboxylic cycle in nerve.

SUMMARY

Na fluoroacetate is almost inactive on frog sciatic nerve. Conduction is unaffected at 100 mM concentration and oxygen consumption is only 50% depressed. Lack of penetration does not adequately account for this feeble activity. Methylfluoroacetate interferes with resting metabolism and leads to irreversible conduction block, large fibers before small ones, after a 'latent' period during which threshold is rising. In summer frogs, more sensitive than winter ones, 5 mM MFA blocks conduction in 3 to 4 hours by raising the threshold 2- to over 5-fold and slowing conduction 2-fold or more. Resting potential and spike height, for individual fibers, are not altered. Resting oxygen consumption is cut to 70 per cent normal by 1 mM MFA, to 20 per cent by 5 mM; but the extra Q_{O_2} of activity may remain intact when the resting value is severely cut. Fumarate and succinate can protect against MFA action; ethanol, acetate, pyruvate, alpha-ketoglutarate, and glucose are ineffective.

These findings emphasize the safety factor in nerve metabolism and the metabolic difference between nerve and muscle, and support the existence and functional importance of a tricarboxylic cycle in nerve metabolism. The selective inhibition of resting rather than of active oxygen consumption, by MFA on nerve, is a unique reversal of the more usual selective inhibition of activity metabolism.

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ACTION OF ANTICHOLINESTERASES, DRUGS AND INTERMEDIATES ON RESPIRATION AND ELECTRICAL ACTIVITY OF THE ISOLATED FROG BRAIN¹

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WAR research has made available a number of new metabolic inhibitors with striking pharmacologic actions. Some of these are potent cholinesterase (ChE) inhibitors, as diisopropyl fluorophosphate (DFP) and the polyphosphonate esters, tetraethylpyrophosphate (TEP) or hexaethyltetraphosphate (HTP), and their action has been widely interpreted in terms of an accumulation of acetylcholine (ACh). Others do not affect ChE, as sodium or methyl fluoracetate (MFA), and their action has been ascribed to interference with oxidative sequences and energy yield. The functional importance of a particular chemical step can be explored by observing the effect on function of blocking that reaction, as in the classical work with IAA on lactic acid formation in muscle. We have, accordingly, studied the action of several of these inhibitors, alone and in combination with eserine, atropine, caffeine or Krebs's cycle substrates, on the isolated frog brain. This preparation yields a regular electric rhythm spontaneously and large recurrent traveling potential waves under the influence of strong caffeine, and these have been taken as an index of the physiological state of the tissue. As a further check on the overall metabolism, the oxygen consumption of bits of brain has been followed. A few assays of ChE inhibition of brain homogenate or slices have also been performed (with D. Luck). The physiological action of both types of inhibitors (MFA, DFP, TEP) has been found to follow more closely their inhibition of oxygen consumption than their inhibition of ChE.

METHODS

Preparation of and recording from the isolated frog brain have followed the earlier description (1). Reagents were applied by soaking in the desired solution for a short time, usually 2 minutes, then dipping quickly into Ringer. When caffeine (0.5%) waves were being studied, caffeine (0.1%) was re-added with the test solution to prevent washing out of the alkaloid.

For respiration studies, the capillary respirometer (2) and the Warburg methods have been used. Bits of the primordium palli, some 2 mm. long oriented from midline to lateral convexity, some 1 mm. wide from anterior to posterior, and 0.2 mm. thick from pial to ventricular surfaces, were used in the capillary. Each bit weighed about 0.7 mg. and experimental and control pieces were taken as far as practicable bilaterally from like regions of the hemispheres. In the Warburg experiments, 3 hemispheres, sliced into pieces roughly of the above size, were used in each manometer. Since, after completing the preparation, some time must elapse before satisfactory readings are

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obtained, QO_2 values are mostly given for the second hour after drug application. The brain bit was left in the desired solution for 2 to 6 minutes (less often 15 to 20, with like results), quickly washed, blotted, and mounted in the respirometer. Readings were normally begun half an hour later. Vapor pressure of the drugs themselves did not alter readings. Electrical measurements were made at room temperature, mostly 20° to 23°C .; respiration measurements in a bath at 25° to 28°C .

ChE assays were made by a standard frog rectus method after incubating ACh with brain homogenate. Manometric measurements supplemented these, 50 per cent inhibition by the various drugs being attained at some ten-fold lower concentrations. Figures are given for the muscle assay.

The drugs were obtained from the University of Chicago Toxicity Laboratory, (Dr. Hutchens), the Victor Chemical Works and Edgewood Arsenal (Dr. Himwich), and were tested for purity by measurement of boiling point. Stock material was kept in a desiccator, solutions being made in Ringer bicarbonate (gm/l. NaCl 6.5, KCl .2, CaCl_2 .25, NaHCO_3 .15), adjusted to a pH of 7.0 to 7.5 before using. Results obtained, especially with TEP, were different quantitatively from one season to another but probably this variance is due to the frogs and not to the use of different batches of chemicals. Other differences, in critical drug concentrations and even type of electrical effect, were encountered when some of these experiments were repeated in the summer at another laboratory. The results reported are those regularly obtained here.

RESULTS

NaFA. This agent was inactive, even in 50 mM concentration, on the normal or caffeine waves of frog brain or on its respiration. This inertness, despite powerful pharmacological action *in vivo* on mammals (2a, 3a), is not due entirely to lack of penetration, for NaFA proved similarly ineffective in slowing MB reduction by rat brain homogenate (3). Similar lack of activity for muscle has been observed (4). On intact peripheral nerve, also, NaFA did not depress conduction or respiration (5), although in other species marked depression of brain respiration has been found (6).

MFA. In contrast to the sodium salt, the methyl ester inhibits respiration by 10 per cent at 5 mM, by 45 per cent at 12 mM and by 80 per cent at 25 mM. At 0.1 to 2 mM, there may be some stimulation of O_2 consumption (table 1 A). MFA also inhibits a number of dehydrogenases, as tested by MB reduction (3), and depresses conduction and respiration of nerve (5). ChE is 50 per cent inhibited at 10 mM. Action on brain potentials begins at 12 mM, which cuts amplitude to half without affecting frequency, the change being complete within 20 minutes (fig. 1). Higher concentrations produce the same changes, but more rapidly, until 100 mM, which abolishes all activity after a 2-minute soaking. After a one-minute exposure to this concentration, a very feeble rhythm of normal frequency remains.

In the presence of .5 per cent caffeine, 12 mM MFA inhibits respiration by only 20 per cent. Caffeine spikes are transiently increased in amplitude and frequency by this MFA concentration, which is threshold, but return to normal in a minute. Stronger solutions (16–50 mM) abolish all activity or cut both amplitude and frequency (to 50% by 25 mM exposed for half a minute, to zero in 4 minutes, exposed for 2 minutes). All the MFA effects on electrical waves can be reversed by prompt washing, but after a few minutes they are permanent.

DFP. At 1 mM concentration, DFP inhibits oxygen uptake by 10 per cent; at 10 mM, by 50 per cent (table 1 B). ChE is inhibited about 50 per cent by 0.3

TABLE 1. INHIBITION OF BRAIN OXYGEN CONSUMPTION

NUMBER OF EXPERIMENTS AVERAGED	DRUG CONCENTRATION (mM)	Q _{O₂} (2ND HR.)		INHIBITION (%)
		Control	Drug	
A. MFA				
1	0.01	440	435	0
1	0.1	370	385	-4
1	2.	755	790	-5
2	5.	880	830	6
3	10.	565	310	45 (40-50)
2	12.	520	300	42 (32-52)
3	16.	365	140	62 (50-74)
1	25.	430	80	81
B. DFP				
1	0.01 ¹	385	390	0
1	0.5	580	330	43
1	1. ¹	305	265	13
1	3. ¹	180	160	12
2	5. ¹	345	235	32 (24-40)
1	6. ¹	240	140	45
1	10.	495	305	38
2	10. ¹	325	170	48 (44-52)
C. TEP				
1	10 ⁻⁶	375	355	5
2	10 ⁻⁶	810	730	11 (5-17)
4	10 ⁻⁴	515	465	10 (5-15)
1	10 ⁻⁴ ¹	385	320	17
2	0.01	330	285	14 (10-18)
1	0.01 ¹	360	305	16
1	0.1	395	335	15
2	1.	325	195	40 (35-45)
2	1. ¹	320	205	36 (33-39)
2	10.	715	340	57 (49-65)
D. ES				
2	10. ¹	340	345	-2 (0- -4)
2	20. ¹	400	290	28 (27-29)
E. AS				
1	0.01	340	340	0
2	0.1	585	555	5 (3-7)
1	0.1 ¹	225	230	-3
4	1.	545	435	20 (9-31)
2	1. ¹	255	255	0 (-4 to +3)

¹ These experiments in Warburg, others in capillary respirometer. Control values average higher in the capillary (525) than in the manometric (325) method; but % inhibition in comparable experiments is alike by both methods. The higher variation in the capillary is probably due to the much smaller samples used; the higher average perhaps to less damage. Some experiments at one concentration are given individually to show the similarity of inhibition despite variation in conditions. Values in () indicate the range of inhibition, which is much less than the range of absolute values from brain to brain.

mm. Spontaneous electrical waves are first affected at 10 mm, which leads to a prompt and marked slowing to 2 to 3 a second, with no initial change in amplitude (fig. 1). Over about 10 minutes, amplitude falls greatly, with spindling recurring at 10-second intervals, but there is no further frequency change.

In the presence of caffeine, 1 mM DFP inhibits respiration during the second hour by 20 per cent; 5 mM, by 35 per cent (table 5 B). Even 0.1 mM markedly increases the after train of caffeine spikes. At 5 mM the caffeine spikes begin to be depressed in amplitude and frequency, and at 10 mM they are abolished by a 2-minute

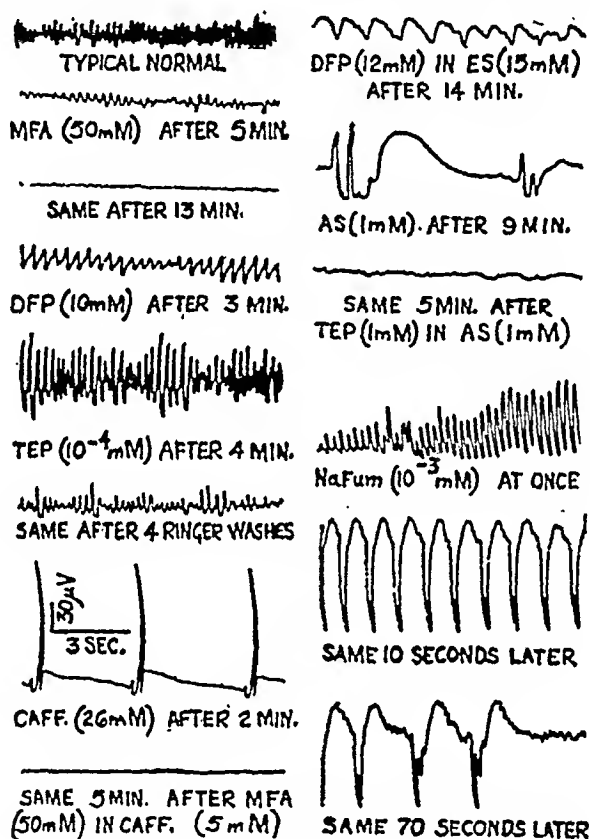


Fig. 1

soaking. All the DFP effects can also be stopped or reversed by washing within a few minutes of drug application.

TEP. Both electrical activity and respiration are affected by this agent at far lower concentrations than by the others. 10^{-5} mM solutions depress respiration by 10 per cent, and even 0.1 mM depresses little more, by 15 per cent. One to 10 mM concentration cuts respiration to half (table 1 C). Three $\times 10^{-4}$ mM inhibits ChE about 50 per cent. The spontaneous electric waves are first altered by 10^{-4} mM TEP, which triples their amplitude without affecting frequency (fig. 1). No further action is obtained by increasing the concentration through the one to 10 mM range, but at 10 mM activity is promptly abolished. (In late spring to early fall frogs only a depression in amplitude appeared, beginning at 10^{-4} mM and being complete at 10 mM.)

In the presence of caffeine (.5%), 1 mM TEP inhibits respiration by 15 per cent,

5 mM by 40 per cent (table 5 B). This is the TEP concentration range in which the effect on caffeine waves changes sharply, being increased in amplitude and frequency for several minutes by 1 mM solutions, depressed by stronger ones. Five mM TEP abolishes caffeine activity over 15 minutes, 10 mM, within a minute or two. Some, but incomplete, reversibility of these effects is obtained by prompt washing.

Eserine Sulphate or Salicylate (ES). ES at 3×10^{-3} mM inhibits ChE about 50 per cent. One to 5 mM ES slows the spontaneous rhythm to half frequency and at 10 mM doubles amplitude as well. Occasionally, regularly-repeated convulsive spikes appear. Similar findings with ES, but at 0.01 mM, and with ACh have been reported earlier (7). The ES action is reversed by washing at 30 to 60 minutes. Ten mM ES does not alter Q_{O_2} , but 20 mM produces a 30 to 40 per cent inhibition, persisting over both the 2- and the 4-hour measurement periods (tables 1 D and 2 A). A control with like concentrations of sodium sulphate or salicylate showed no inhibition. These data were especially needed for study of the combined action of ES and more irreversible anticholinesterases.

Several workers have shown that prior treatment with ES and related drugs will protect against DFP action (8, 9, 48; but not in the roach, 10), and recent evidence (34) is clear that ES can protect rats against several LD_{50} 's of DFP without preventing the almost complete inhibition (98-99%) of ChE. Block of nerve conduction by DFP can similarly be prevented by ES, ChE in this case being also protected from inhibition (11).

DFP or TEP (each 6 mM) inhibits frog brain Q_{O_2} in the presence of (and after 2 hours pretreatment with) ES (20 mM) to a deeper level than in the absence of ES—65 per cent inhibition versus 40. ES does not, therefore, fully prevent the action of these drugs, although ChE must have been entirely inhibited by the ES; but the effects on Q_{O_2} are not additive. A more searching experiment consisted of exposing hemispheres to ES (12 mM) for 15 minutes, then to DFP (3 or 6 mM) in ES for 15 minutes, then washing 5 times in Ringer over a further 40 minutes. Their Q_{O_2} 's were compared with those of hemispheres similarly exposed to DFP alone or merely to Ringer. The results (table 2 B) show a clear protection by ES against DFP inhibition and a lesser protection against TEP. During the first 2 hours of measurement, 85 per cent of the DFP and 35 per cent of the TEP inhibition is prevented. A comparable experiment on sciatic nerves (6 nerves, about 120 mg. per Warburg vessel) gave like results (table 2 B). DFP (3 mM) alone gave 35 per cent inhibition during the first hour, ES-DFP gave no inhibition, thus protecting 100 per cent. On the electrical side, also, ES can protect against DFP abolition of spontaneous waves. DFP (10 mM) alone slows the waves and then cuts them down and it at once abolishes the slow ones present after ES (2 mM). But in the presence of 15 mM ES, the slow waves continue for half an hour with DFP.

In both DFP and ES experiments, Q_{O_2} values for the third and fourth hours were higher for the drugged brain or nerve than for the controls. The reasons for this late change in inhibition (destruction of drug, e.g. 12, preservation of more substrate, etc.) and in protection have not been explored.

Atropine Sulphate (AS). If the effects of these anticholinesterase drugs were due to ACh accumulation, atropine might be expected to prevent them. (There

is much disagreement as to the extent of prevention of ACh, DFP, and TEP action by AS, see 27 and review 53.) It was earlier reported (7) that ACh, like eserine, pro-

TABLE 2. ES AND DFP OR TEP ON BRAIN AND NERVE RESPIRATION

A. Combined Inhibition: Brain.

TIME, MIN.	ADDITION, Q_{O_2}		INHIBITION, %	ADDITION, Q_{O_2}		INHIBITION, %	ADDITION, Q_{O_2}		INHIBITION, %
	Ringer	ES ¹		Ringer	ES		Ringer	ES	
0-240									
0-15	415	430		430	445		395	400	
60-120	300	180	40	320	235	27	305	200	35
120-240	Ringer	Ringer		DFP ²	DFP		TEP ³	TEP	
180-240	240			140	80		145	80	
% inhibition ³		52		43	67		40	67	
% inhibition ⁴					30			30	

B. Protection by ES Pretreatment (see text)

TISSUE	RINGER (Q_{O_2})	R DFP (3mM) R (Q_{O_2})	INHIBITION, %	ES ⁵ DFP (3mM) R (Q_{O_2})	INHIBITION, %	R DFP (6mM) R (Q_{O_2})	INHIBITION, %	ES DFP (6mM) R (Q_{O_2})	INHIBITION, %	R TEP (5mM) R (Q_{O_2})	INHIBITION, %	ES TEP (5mM) R (Q_{O_2})	INHIBITION, %
First Hour													
Brain	445	315	29	435	2								
	270					240	11	255	5				
	190									125	34	145	24
Nerve	100	75	25	105	0								
Second Hour													
Brain	375	300	20	350	7								
	200					120	40	205	0				
	175									75	57	110	37
Nerve	85	55	35	55	35								
Third Hour ⁶													
Brain	305	225	26	265	13								
	140					100	29	90	36				
	135									60	56	85	37
Nerve	75	65	12	50	33								

¹ ES = 20 mM in Ringer. ² DFP and TEP = 6 mM in Ringer. ³ At 180-240 min. Compared to Ringer- Q_{O_2} 240. ⁴ At 180-240 min. Compared to ES- Q_{O_2} 115. ⁵ ES 12 mM throughout. Salicylate used with DFP, sulphate with TEP. ⁶ Fourth hour with TEP.

ES present with DFP or TEP in all cases when present before.

duced increased electrical activity of the frog brain and that this was not altered by atropine. On the metabolic side, surprisingly little attention has been accorded ACh. A questionable increase in the Q_{O_2} of eserinated frog brain by ACh addition was found by Lipton (reported in 13).

AS (1 mM), after 3 to 5 minutes soaking, may inhibit brain Q_{O_2} by 15 to 20 per cent (table 1 E), measured after an hour of equilibration and on small bits. Exposure to TEP (.01 mM, 5 minutes soak) subsequent to AS led to a greater inhibition of respiration (45%) than exposure to TEP alone (15%). With stronger TEP (1 mM), inhibition with or after AS (1 mM) could exceed 95 per cent (table 3). Results with DFP and AS were comparable; inhibition of respiration by DFP alone

TABLE 3. AS AND TEP OR DFP ON BRAIN RESPIRATION

TIME, MIN.	ADDITION, Q_{O_2}		INHIBITION, %	ADDITION, Q_{O_2}		INHIBITION, %	ADDITION, Q_{O_2}		INHIBITION, %
0- 5 5- 10	Ringer Ringer	Ringer AS 1 mM		Ringer TEP .01 mM			AS 1 mM TEP .01 mM		
60- 90 60- 90	680 510	520 410	24 19	570 440	16 14		390 300	43 41	
0- 5 5- 10	Ringer Ringer			Ringer TEP 1 mM			AS 1 mM TEP 1 mM		
60- 90	495			285	43		10-	98+	
0- 90 30- 90 90-210	Ringer 255 Ringer						Ringer 265 AS 1 mM, TEP 1 mM		
90-150 150-210	305 220						225 105	26 52	
0-120 0- 60 60-120	Ringer 235 200			Ringer 215 215			AS 1 mM 230 205	0 0	
120-300 180-240 240-300	Ringer 180 215			DFP 3 mM 160 205	11 5		DFP 3 mM 125 170	30 21	

First two experiments in capillary respirometer, others in Warburg.

being under 10 per cent, by DFP after atropine, 25 per cent (table 3). On the electrical side, 1 to 10 mM AS slows the spontaneous rhythm by 30 to 50 per cent and progressively cuts amplitude to zero in 10 minutes. Convulsive spikes appear, however, and grow in size (to 250 μ V) while decreasing in frequency (to 1 in 6 sec.) over a 30-minute period (fig. 1). These spikes are not abolished within 5 to 10 minutes by amounts of DFP and TEP which abolish the usual spontaneous waves at once. Concentrations of AS which do not lead to spiking do not alter the electrical effects of TEP, over the range 10^{-5} to 1 mM.

Dicarboxylic Acids. Fumarate, particularly, is able to counteract the inhibition of oxygen consumption and block of conduction induced in nerve by MFA (5). We have made comparable experiments on brain and with other inhibitors. Sodium fumarate (16 mM), present before addition of MFA (11 mM) from a side arm, prevents 75 per cent of the usual inhibition of Q_{O_2} produced by MFA alone (table 4).

TABLE 4. PROTECTION BY FUMARATE OF BRAIN RESPIRATION

TIME, MIN.	ADDITION, Q_{O_2}		INHIBITION, %	ADDITION, Q_{O_2}	INHIBITION, %
0-120	Ringer	Ringer		Fumarate 16 mM	
60-120	440	460		450	
120-240	Ringer	MFA 11 mM		MFA 11 mM	
120-180	375	290	23	360	4
180-240	340	205	40	305	10
0-60		Ringer		Fumarate	
0-60		295		310	
60-300		DFP 6 mM		DFP 6 mM	
60-120		220		270	
120-180		180		225	
180-240		160	(43) ¹	155	(29) ²
240-300		110		105	
0-60		Ringer		Fumarate	
0-60		370		380	
60-300		TEP 1 mM		TEP 1 mM	
60-120		290		325	
120-180		250		265	
180-240		240	(40) ¹	265	(34) ²
240-300		205		240	

¹ From experiment II A, run under parallel conditions.

² Calculated on basis of interpolated inhibition.

The fumarate itself perhaps increased respiration slightly, 2 to 3 per cent. In comparable experiments, fumarate (16 mM) gave a 30 per cent protection against the inhibition of oxygen consumption by DFP (6 mM), half this protection against TEP (1 mM) inhibition (table 4).

Attempts to explore the protective action of fumarate on the electrical effects of the experimental drugs were confounded by an entirely unexpected action of fumarate itself. (Fumarate was finally demonstrated to prevent the electrical changes, as well as most of the respiration inhibition, induced by MFA.) Even at

0.001 mM concentration, soaking for 3 minutes in Na fumarate leads to a dramatic change in the spontaneous brain potentials. The waves become progressively larger and slower until convulsive-type swings of 200 μ V at one per second are reached. The brain then becomes abruptly silent for minutes, when the whole sequence may repeat or stop for good (fig. 1). Similar effects, but at rather greater concentration, are produced by glutamate (1 mM), α ketoglutarate (5 mM), oxaloacetate (10^{-2} mM), aspartate (10^{-2} mM), and succinate (1–10 mM). Valine, pyruvate and glucose, on the contrary, are entirely inert. The action appears to be associated with the presence of the 4 to 5 carbon dicarboxylic skeleton. These results were obtained consistently in many experiments during the winter of 1948 at Chicago. Attempts to repeat them the following summer were negative, no effect of these substrates being observed. Several have again been obtained here this winter (but fumarate only at 10 mM or above) plus the further finding that when a brain has become silent after, say, fumarate, it can respond well to, say, aspartic and after this will respond again to fumarate. We cannot now account either for the phenomenon or its variability. Sex and temperature at which the frogs are kept are immaterial; different batches of chemicals, with or without recrystallization, have behaved alike. If impurities are involved, they must be extremely potent. The marked variation in blood sugar of frogs, from 75 mg. % in summer to 200 mg. % in winter (11a) deserves note; as also a seasonal difference in drug sensitivity (12a, 13a).

Caffeine. Since this drug was used (as alkaloid) regularly in the electrical experiments, a few tests were run on its influence on respiration. Exposure for 2 to 3 minutes to 0.5 per cent caffeine (26 mM) increases oxygen consumption by a third, as it also increases the brain's electrical activity; and even greater increases, up to doubling, are obtained on long exposure to 0.1 or 0.25 per cent caffeine. Similar long exposure, one or two hours, to 0.5 per cent caffeine inhibits respiration 15 per cent, and also abolishes all electrical activity (table 5 A). DFP, added with 0.5 per cent caffeine, does not increase the caffeine inhibition when in a concentration of 1 mM, which increases caffeine waves, but does inhibit further (doubles the inhibition) in a concentration of 5 mM, which depresses caffeine waves. MFA, 12 mM, or TEP, 1 mM, does not increase the caffeine inhibition, but 5 mM TEP, which depresses caffeine waves, does (table 5 B).

Nerve. A few experiments on frog sciatic nerve respiration (by K. S. Crippen) yielded results similar to those on brain. One to 4 runs each were made in duplicate with each agent, compared to Ringer controls, on 5-mg. pieces of nerve in the capillary respirometer. Nerves were soaked for 1 to 2 hours (all solutions at pH 6.9–7.3) before mounting and then followed for 60 to 90 minutes. At 10 mM concentration, neither fumarate or succinate increased oxygen consumption, nor did 30 mM malonate inhibit. TEP, 10^{-3} mM, gave a 40 per cent inhibition; DFP, 5 to 20 mM, a 60 per cent inhibition; and ES, 20 to 40 mM, a 25 per cent inhibition. Our findings are summarized in table 6.

DISCUSSION

Evidence has been offered that the fluoroacetates specifically inhibit acetate oxidation, presumably due to competitive inhibition by the halogen compound of appro-

TABLE 5. CAFFEINE ON BRAIN RESPIRATION
A. Alone

TIME, MIN.	ADDITION, Q_{O_2}		INCREASE, %
0-10	Ringer	Caffeine 0.5%	
0-60	565	585	3
0-60	570	755	24
0-60	585	840	41
0-240	Ringer	Caffeine 0.5%	
0-60	330	235	-28
120-180	170	145	-15
180-240	155	130	-17
0-120	Ringer	Caffeine 0.25%	
0-60	340	430	26
60-120	265	395	49
0-180	Ringer	Caffeine 0.1%	
0-60	510	575	13
60-120	440	455	4
120-180	360	270	-27
0-60	200	375	88
60-120	100	275	175

B. With Other Drugs

TIME, MIN.	ADDITION, Q_{O_2}		INHIBITION, %	
0-240	Ringer	Caffeine 0.5%		
0-30	650	590	9	
60-120	360	300	17	
120-240	Ringer	DFP 1 mM DFP 5 mM		
120-180	365	320 230	12	37
180-240	260	200 180	23	31
0-120	Ringer	Caffeine + MFA 12 mM		
60-120	560	455	20	
0-120	Ringer	Caffeine + TEP 1 mM		
60-120	635	505	21	
60-120	415	360	13	
0-120	Ringer	Caffeine + TEP 5 mM		
60-120	375	215	43	

TABLE 6. SUMMARY

NO. ELECT. EXPER.		DRUG	CONC. (mM)	ACTION ON FROG BRAIN (NORMAL LEVEL = 1.0)							
				Electrical activity				Q _{O2}			Ch. E.
				Spont. rhythm		Caff. waves		Brain		Nerve	Brain
				Ampl.	Freq.	Ampl.	Freq.	Normal	Caff.		
9	9	NaFA	50	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
24	20	MFA	5	1	1	1	1	0.9		0.2	
			12	0.5	1	1 \rightarrow 1	1 \rightarrow 1	0.5	0.8		0.5
			25	0.5	1	0.3 \rightarrow 0	0.3 \rightarrow 0				
			50	0.5	1	0	0				
			100	0	0	0	0	0.2			
10	17	DFP	10 ⁻¹	1	1	1 \rightarrow 1	1	1.0			
			0.3	1	1	1 \rightarrow 1	1	1.0			
			1	1	1	1 \rightarrow 1	1 ²	0.9	0.8		0.5
			5	1	1	0.5	0.6				
			10	1 \rightarrow 0.2	0.5	0	0	0.5		0.4	
47	20	TEP	10 ⁻⁶	1	1	1	1	0.9			
			10 ⁻⁴	3	1	1	1	0.8			
			3 \times 10 ⁻⁴	3	1	1	1	0.8			
			10 ⁻³	3	1	1	1	0.8		0.6	0.5
			10 ⁻¹	3	1	1	1	0.8			
			1	3	1	1 ⁺	1 ⁺	0.6	0.8		
			5	3	1	0.5 \rightarrow 0	0.6 \rightarrow 0	0.6	0.6		
			10	0	0	0	0	0.5			
23	5	ES	3 \times 10 ⁻³	1	1			1.0			
			1	1	0.5			1.0			
			5	1	0.5			1.0			
			10	2	0.5			1.0			
			20					0.6		0.8	
13		AS	1	1 \rightarrow 0	0.5 ³			0.8			
			10	0	0			DFP, TEP synergize			
7	7	ACh	10 ⁻³	1	1	5	0.5				
			1	1	1	1	0.5				
			10	0.2 \rightarrow 0	1 ⁴	0	0				
10		Caff.	5	1	1			2			
			25	1 \rightarrow 0	1 \rightarrow 0 ⁵			1.3 \rightarrow .8 ¹			

¹ DFP or TEP depressed Q_{O2} when waves depressed. ² caffeine train increased. ³ spikes not abolished by DFP or TEP. ⁴ spikes appear. ⁵ spikes and train appear.

priate dimensions to fit the active enzyme (6, 14). Other work (15, 16, 16a) has emphasized an action on the oxidative decarboxylation of pyruvic acid and has thrown

doubt on the competitive nature of the inhibition. Our finding on brain, as well as comparable ones on nerve (5), brain brei (3), and muscle (4) that MFA is far more potent than NaFA, also speaks against a precise steric interaction of the fluoroacetate radicle with the enzyme. All workers (14, 16, 17, 18) offer evidence of interference with the formation or oxidation of succinate or citrate (18a, 19a) and most believe that the tricarboxylic cycle is interfered with, whether decarboxylation or a splitting of active acetate (14) occurs at the oxidative steps. The present findings on brain and comparable ones on nerve (5), that MFA inhibition of respiration and of function can be overcome by several tricarboxylic cycle intermediates, notably fumarate, also strongly suggest an action on the Krebs cycle. We would particularly suspect the step from alpha ketoglutaric to succinic except for the finding on muscle (17) that MFA inhibits succinic but not alpha ketoglutaric oxidation. Our results, then, favor the existence and functional importance in brain (and nerve) of the Krebs cycle. No one has suggested that MFA affects ChE, *in vitro* evidence (6) being to the contrary except for an experiment here in which 20 mM MFA inhibited one-third of the ChE activity of frog brain on a 30-minute incubation *in vitro*.

DFP, TEP, and HTP, in contrast to MFA, are widely recognized as ChE inhibitors, and fluorophosphonates have been found not to inhibit a variety of other enzyme systems *in vitro* (19). It is commonly assumed, therefore, that these substances produce functional changes by their action on the ACh system. MFA, however, was likewise found (6) inactive on 18 purified enzymes, including the very ones that are clearly affected *in vivo*; and comparable cases of insensitivity of purified systems are well known (20). DFP, TEP and eserine have now been shown to inhibit oxidations of frog nerve, or rat brain brei, and, in the present experiments, of frog brain slices, in concentrations comparable to those which are physiologically active or which inhibit ChE (e.g. eserine on nerve, 21-24). DFP and TEP, at 0.1 mM, were also observed in other experiments to inhibit the growth of *Lactobacillus arabinosis* (C. Haber). Furthermore, although some work (25-28, 30-32) favors the conclusion that symptoms and death produced by anticholinesterases run parallel with the degree of inhibition of brain ChE, (although the level of the ChE at which death appeared varied in different reports between 0 [30, 36] and 95% [35] normal), other recent studies (32-34) trend in the other direction. High ChE activity, over 80 per cent normal, may be present in the brains of rats dying from ES (35) and low ChE activity (15%), in the brains of survivors (34, see also 32). Further, rats pretreated with ES and surviving 2 to 4 times the LD₅₀ of DFP, consistently show less than 2 per cent of the normal ChE activity of cortex or caudate or, with larger ES and DFP doses, followed hour by hour, become symptom-free while the ChE is still falling (at 10-15% normal activity). Accumulation of high ACh concentrations in the brain support the evidence that ChE is inhibited *in vivo* (34). Comparable results on ChE are reported for monkeys (36); see also (37). It has even been found that when ChE is fully inactivated to ACh by ES its activity on another substrate (chloroacetic ethyl ester) is unimpaired (38). The literature is conflicting enough, even on whether true ChE is as sensitive as pseudo ChE to these drugs (36, 39) or not (38, 27, 40, 36, 31, 19) so that a summary is dangerous; but table 7 cannot be far off in order of magnitude.

From table 7 the following rough statements can be made. All nerves tested

(invertebrate giants, mammalian sympathetic, frog and fish somatic) block within an hour when exposed to any of the antiesterase drugs in the concentration range of 10 to 20 mM. (DFP on roach giant is 60 mM.) TEP is somewhat more active in general, ES and DFP change positions from case to case. In DFP-blocked nerves, the ChE is found to about 80 per cent inhibited by 3 workers, 92 or 95 per cent in 2 other papers; in nerves conducting fully, the ChE inhibition is reported by 3 groups to be 100, 98, and 93 per cent inhibited. The concentration of a given drug in a given species required to block nerve conduction is 1,000- to 100,000-fold greater than that needed to alter central nervous function. Brain ChE of mammals is 50 per cent inhibited *in vitro* by TEP or DFP at a concentration range centering on 10^{-5} mM (but some reports put DFP concentrations at 10^{-3} or higher); by ES at 10^{-3} . Frog brain is less than one-hundredth as sensitive to ES as is mammalian brain (50% inhibition at 10^{-3} and 10^{-5} , respectively) in comparable experiments; roach cord is as sensitive or more so to ES (50% inhibition at 10^{-5} mM) and less sensitive to TEP or DFP (50% inhibition at 5×10^{-4} and 5×10^{-2} , respectively) than is mammalian brain. The *in vitro* drug concentration required for altered central nervous function and for 90 per cent ChE inhibition is often similar but may differ by a power of 4 (ES on roach cord). *In vivo* administration to mammals indicates that TEP is more lethal than DFP (LD_{50} : TEP, about 0.002 mM/K; DFP about 0.02) and there is the same disagreement as for nerve as to whether death parallels ChE inhibition (50-100% inhibition reported in fatalities at LD_{50} ; 75% to over 90% in survivors—even at LD_0 with DFP). Since lethality may depend on particular sites of action, central or peripheral, and drug penetration may complicate *in vivo* studies (34, 35), such disagreements might be expected. It seems clear, none the less, that there is inadequate justification for attributing the neural effects of the 'anticholinesterase' drugs primarily to their inhibition of ChE and an attendant accumulation of ACh.

It is, in fact, unlikely that all these drugs act even on the same link in the metabolic chain, at least in the same neurones; for if so their effects should be essentially identical and other agents should alter these in similar fashion. Yet DFP first slows the normal electrical waves without changing their amplitude, while TEP mainly increases and MFA decreases their amplitude without altering their rate. Caffeine waves are unchanged in frequency, except for a transient speeding up by MFA, while all 3 drugs increase their amplitude, at least transiently, at appropriate concentrations. Caffeine, itself reported to inhibit ChE (54, 60, and, at higher concentrations, succinic dehydrogenase, 55), produces entirely different electrical effects. These various drugs do not, therefore, act alike, as if each inhibited ChE specifically; rather all produce somewhat unique functional changes, as if each tangles with the metabolic belt in its own way. This is further indicated by the inability of TEP and MFA or of TEP and ACh, each present in half that concentration needed to stop electrical activity, to sum their effects. In such mixtures, activity continues. Further, ES protects respiration and electrical activity against DFP doses that would normally stop brain waves at once; while AS does not protect against DFP (both results occur in mammals; 49, 57, 53 and references)—it actually enhances the depression of respiration by DFP and TEP—nor do these drugs prevent the electrical effects of AS itself. There is, thus, no evidence for a common action on the ACh-ChE system.

It is noteworthy, though, that these drugs (except ES), with or without caffeine,

TABLE 7. SUMMARY OF LITERATURE

A. Nerve

ANIMAL	NERVE	DRUG	IN VIVO		IN VITRO			
			Conc. mm/K	LD	Conc. (mm)	% ChE inhib. in nerve	Conc. (mm)	Physiol. effect
Roach	Giant fiber	HTP	.01	50(26)			7(25)	Block
		DFP	.03	50(26)			60(25)	Block
Squid	Fin	DFP			13	80 (41)	13(41)	Block in 30 min.
Earth- worm	Giant fiber	DFP					25(21)	Block in 4 min.
Cat	Cervical symp.	DFP			20	80 (23)	20(23)	Block in 2-5 min.
	Tibial	ES					20(23)	Block in 30 min.
		DFP					20(22)	Block in 20 min.
Skate	Optic	DFP					15(43)	Block in 4 min.
Frog	Sciatic	DFP	11.			98 (22)	(22)	No block
					3	100 (44)	15(44)	No block
					3	93 (24) ¹	3(24)	No block
							20(21, 44, 23, 22)	Block in 20 min.
		E(S)					20(23, 22) 50(21)	Block in 20 min. Block in 70 min.

B. Brain

ANIMAL	DRUG	IN VIVO ²				IN VITRO			
		Conc. ³ mm/K	LD	% inhib. brain ChE		Conc. mm	% ChE inhib. in brain	Conc. mm	Physiol. effect
				Survivors	Fatalities				
Roach ⁴	HTP	.01	50(26)			2×10^{-4}	50(26)	5×10^{-4} (10)	Synapse change
	DFP	.03	50(26)			10^{-2}	50(26)	.6 (10)	Synapse change
	ES					10^{-5}	50(26)	.2 (10)	Synapse block
Frog	ES					10^{-3}	50(56)		
Man	DFP					10^{-3}	50(36)		
Mon- key	DFP	.001	50(40)			3×10^{-3}	50(36)		
		.0005		100(36)	100(36)				

TABLE 7—Continued

ANIMAL	DRUG	IN VIVO ²				IN VITRO			
		Conc. ³ mm/K	LD	% inhib. brain ChE		Conc. mm	% ChE inhib. in brain	Conc. mm	Physiol. effect
				Survivors	Fatalities				
Rabbit	DFP	.02	50(40)	75(30) 75(30)	100(30)				
Dog	HTP	.002	50(27)		60(27) ⁶				
		.01	0(27) ⁸	96(27)					
	DFP ES	.02	50(40)			10 ⁻¹ 10 ⁻⁵	50(37) 50(56)		
Cat	HTP	.001						(52)	N-Minc.
		.004						(52)	NM block
	DFP	.01-.02	50(48, 40)					.02 (53) .02-.07 (48)	EEG change NM block
Mouse	HTP	.01	50(27)						
		.006	0(27)	33(27) ⁷					
	TEP	.003	50(32)						
	DFP	.02	50(40)						
Rat	HTP	.005	50(50)		50(50)	10 ⁻⁵	50(50)		
		.02	50(8)						
	TEP	.002	50(32)	33(32)	72(32)	4-6 × 10 ⁻⁶	50(28, 32)		
		.004	50(28)						
	DFP	.01	50(51, 37, 40)			6 × 10 ⁻⁵	50(50)		
		.05	50(28)			4 × 10 ⁻⁴	50(28)		
		.005	0(37)	90(37) ⁸					
	ES	.007	100(35)		30(35)	10 ⁻⁴ 3 × 10 ⁻²	60(35) 50(37)		
						10 ⁻³	50(31)		
	PS ⁹	.006	100(35)		5(35)	10 ⁻⁴	40(35)		

¹ In various papers, these workers have reported that nerve block occurs when ChE inhibition is greater than the critical value of: 80% (42), 92% (23, 30), or 95% (24).

² Other findings: For all four antiesterases, brain ChE is 50% inhibited at the LD₅₀ (28); but (33) brain ChE is inhibited alike in fatalities and survivors with DFP or ES. DFP symptoms parallel the brain ChE inhibition (51); but symptoms begin at 20-30% ChE inhibition (31).

³ These figures are calculated from mg/K. Route of injection was not alike in all. ⁴ Ganglionated cord. ⁵ Protected by atropine. 96% ChE inhibition at 3 hours; 65% at 20 hours.

⁶ It is reported that HTP death occurs at 80-90% ChE inhibition (27). ⁷ Killed at 15 min. At 2 hours, inhibition is 26%. It is reported (32) that at LD₅₀ brain ChE inhibition with HTP averages 50% (range 25-75). ⁸ At 5 to 24 hours. At 10 days, 55% inhibition. Other workers (33) report the same ACh concentration in brains of DFP survivors and fatalities. ⁹ PS = Prostigmine methyl sulphate.

inhibit the over-all oxygen consumption by 20 to 50 per cent at the concentration, in each case, which provokes functional changes—from 10^{-4} mM in the case of TEP to 12 mM in the case of MFA; and, where tested, they inhibit oxygen consumption by 40 to 50 per cent at the concentration which abolishes electrical activity. Further, from concentrations of DFP and TEP which augment caffeine waves to those which depress, there is a sharp decrease in respiration. The tempting possibility that ChE is involved in normal respiration (e.g. in relation to the choline of lecithin) is excluded by the ability of ES to inhibit ChE without altering oxygen consumption. Others (10, 33, 9, 45, 46) have also noted different effects of different 'antiesterases,' even on the EEG (47, 59), and suspected actions other than ChE inhibition (21, 58). The ratios of concentrations for the various 'antiesterases' for critical action on various systems or species are so widely different as also to indicate other actions of these drugs.

SUMMARY

The isolated frog brain (and nerve) has been used to study the influence of a number of substances on function, indicated by normal and caffeine-induced electrical waves, and on metabolism, indicated by oxygen consumption and cholinesterase activity. The fluoroacetates (NaFA, MFA), inhibitors of carbohydrate metabolism, and the fluorophosphates (DFP), polyphosphate esters (TEP), eserine, and atropine, recognized as acting on the ACh-ChE system, affect function and metabolism in comparable ways, not in different ones. At concentrations in each case (except ES) which alter electrical activity, respiration is inhibited 20 to 50 per cent (about 50% when electrical waves are abolished) but ChE inhibition may be absent or nearly complete. Results with these drugs, caffeine, and ACh are summarized in table 6.

There is no summation of the actions of TEP and MFA or of TEP and ACh. Eserine can largely or fully protect brain and nerve from DFP depression of both function and respiration. Atropine does not protect against TEP or DFP nor is its own action prevented by them. Caffeine in small doses increases electrical activity and oxygen consumption, in larger doses depresses both. Inhibitors combined with caffeine similarly increase or decrease function and respiration in parallel at appropriate concentrations.

Fumarate can protect against MFA action and it, with several other dicarboxylic acids at low concentration—glutamic, α -ketoglutaric, oxaloacetic, aspartic, and succinic (valine, pyruvate, and glucose are inert)—is able to initiate large convulsive potentials in the isolated brain. These effects have been variable.

Our evidence favors a significant role of the tricarboxylic cycle in brain metabolism, one related to the maintenance of function. The present results on frog brain and nerve, as well as those in the literature on rats and other animals, indicate that DFP and TEP disturb function by mechanisms independent of the simple inhibition of ChE. An interference with oxidative metabolism is indicated.

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RESPIRATORY FUNCTION AND BLOOD FLOW IN THE BRONCHIAL ARTERY AFTER LIGATION OF THE PULMONARY ARTERY

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IT HAS long been known that the blood arriving by way of the bronchial arteries is in itself sufficient to support the life of the lung, provided that there is not an excessive back pressure in the pulmonary veins (1-3). Schlaepfer (3, 4) has demonstrated that such a lung, deprived of its pulmonary arterial supply, suffers little histological change, even during a two-year interval. It merely becomes reduced to three-fourths or two-thirds of its usual size and there is slight fibrosis about the bronchovascular rays and in the walls of the alveoli. In these lungs there has been observed grossly an enlargement of the bronchial arterial channels, as well as the development of collaterals from the esophageal and other vessels of the mediastinum (2, 3, 5, 6).

That blood brought to the lungs by way of such arterial channels can enter the capillaries of the alveoli and perform a respiratory function is implied by the existence of instances of pulmonary atresia in man, where branches of the descending aorta constitute the sole arterial supply of the lungs and maintain life for many years (7, 8). Actual indirect measurements of the volume of blood arriving by way of collateral channels in cases of pulmonic stenosis have recently been made by Bing and his associates (9). Anatomical studies of bronchovascular casts of the lungs also have demonstrated the enormous collateral bronchial arterial circulation that may exist in congenital pulmonic stenosis (10) and in chronic bronchiectasis (11). The possible rôle of this systemic collateral in shunting blood from points of anastomosis within diseased tissue to healthy pulmonary parenchyma has been discussed (11). In the intact lung of the dog and of man precapillary anastomoses between the bronchial and pulmonary circulations do not normally exist (12), but blood may reach the capillaries of the alveoli from the bronchial vessels if the pressure in the pulmonary artery is reduced (13).

The present experiments were designed to test, in the dog, in what measure a lung whose main pulmonary artery has been ligated can carry on the function of respiration, and to determine whether such a respiratory function may increase with time. Anatomical studies of the lungs of these animals are reported elsewhere (6).

METHODS

The posterior operative approach to the left pulmonary artery through the fifth interspace was used. This was advantageous in comparison with the anterior ap-

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proach in that a longer segment of the artery could be visualized and isolated for ligation, and retraction of the pulmonary vein was less difficult.

For the bronchospirometric studies a cannula was constructed somewhat on the pattern of the instrument previously described by Van Allen and Lindskog (14). This provided a convenient means of cannulating the right and left halves of the respiratory tree separately. The new cannula consists of two parts: A narrow tube (the left bronchial cannula) 51.5 cm. long and 7.5 mm. in external diameter, which fits within another tube (the tracheal cannula) 31 cm. long and 13 mm. in diameter. When the latter is introduced into the trachea, the distal end of the bronchial cannula can be placed under direct vision into the left main bronchus whose respiratory exchange is thus isolated. The short tracheal tube carries the exchange of the right lung, which is brought out by means of a side arm 7 mm. in internal diameter. The tracheal tube is surrounded by a collapsible rubber cuff to insure an airtight intra-

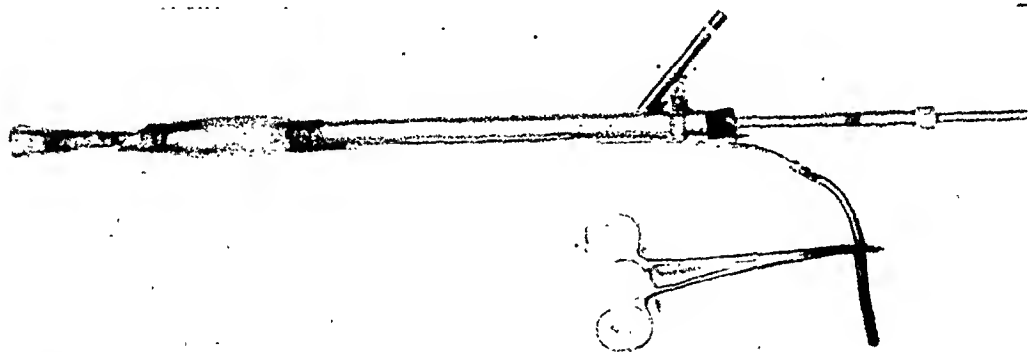


Fig. 1. BRONCHOSPIROMETRIC CANNULA. The Van Allen bronchial unit is shown held in place within the tracheal cannula. In practice the rubber stopper is not inserted until the bronchial unit has been fixed in the left main bronchus. The screw thread, manipulation of which spreads the rubber covered petals of the bronchial extremity, is at the right. The side arm connecting to the lumen of the outer, tracheal, unit projects obliquely upwards. The bag that assures a tight tracheal seal has been inflated. In use, a pressure bulb and mercury manometer would be attached to the rubber tube that is shown clamped.

tracheal seal. The inner bronchial element is held in place with a rubber stopper coated with vinylite after it is seated, to prevent leakage of gases. The bronchial element has a larger lumen than Van Allen's original cannula, to reduce the factor of obstruction. This element consists of two closely fitted tubes which are joined at the external end by a screw thread (figs. 1 and 2). By use of this thread the inner ends of the tubes can be separated or brought together. The outer sleeve has at its bronchial extremity four hinged petals, and the inner ends in a cone. When the inner tube is unscrewed the cone wedges the petals apart. These petals are covered with a rubber membrane to make an airtight seal with the wall of the bronchus.

The bronchospirometric test was performed after the dogs were anesthetized with sodium pentobarbital, 35 mg/kg. intravenously. Under direct vision the instrument was passed through the larynx until both tubes extended well into the trachea. A bronchoscopic light carrier was then introduced into the inner tube and the instrument was further advanced until the carina was visualized. The inner tube was made to enter the left main bronchus. The cannula was now fixed in place

by screwing the knob counter-clockwise, thereby opening the petals on the end. The rubber stopper was placed around the smaller tube, fitted snugly into the larger tube, and sealed off with vinylite. The tracheal bag was inflated and kept at approximately 100 mm. of mercury under manometric control.

Inspection of the cannula *in situ* at necropsy in a series of dogs demonstrated the utility of the instrument and the airtight seal at the left stem bronchus. To test for leaks around the inner end of the left bronchial cannula in living animals, a 'smoke test' was found useful. Cigarette smoke was blown into the left lung through the inner tube. Its failure to appear on the right side and maintenance of positive pressure after a few respirations was taken as evidence against the existence of a leak. When the absence of leaks was demonstrated each lung was connected to a recording spirometer. Any lag, inspiratory or expiratory, of one spirometer behind the other indicated partial obstruction and necessitated readjustment of the cannula.

Fig. 2. CLOSE-UP OF THE VAN ALLEN BRONCHIAL CANNULA IN OBLIQUE VIEW. It shows the petals covered with rubber. Retraction secured by manipulating the screw thread shown in fig. 1, of the pyramidal tip of the inner element spreads the petals and assures a tight connection with the lumen of the bronchus.



Two Benedict-Roth spirometers were used, one for each lung, employing a closed-circuit with flutter valves and soda lime carbon dioxide absorber between expiratory valve and spirometer. Both spirometers recorded simultaneously by means of inked pens on the same drum.

Blood specimens were drawn in oiled syringes containing a minute quantity of heparin powder, mercury sealed and capped, and chilled immediately in water containing ice. Analyses of carbon dioxide and oxygen content and carbon monoxide capacity were determined manometrically according to the standard methods in Peters and Van Slyke (15). Gas samples were collected over mercury and analyzed in the Henderson-Haldane apparatus.

PROCEDURE

The oxygen uptake of the left lung and the oxygen content of the arterial blood were measured under the following conditions:

- I. *Both lungs breathing air.*
- II. *Left lung breathing oxygen, right lung breathing air.*

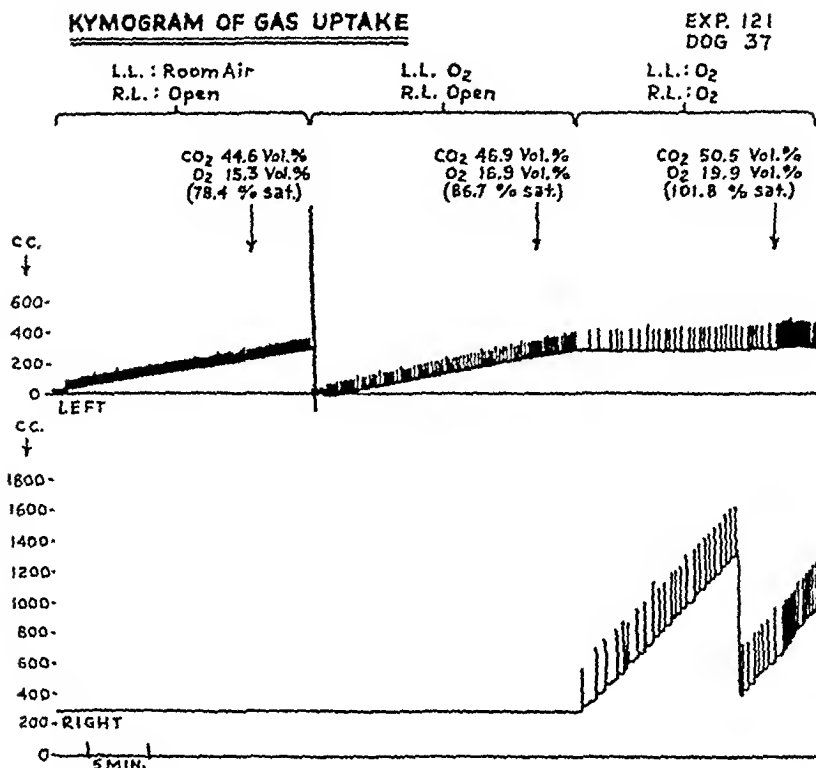


Fig. 3. A. UPPER GRAPH: Left lung. B. LOWER GRAPH: Right lung. Phase I at the left, phase II in center and phase III at right.

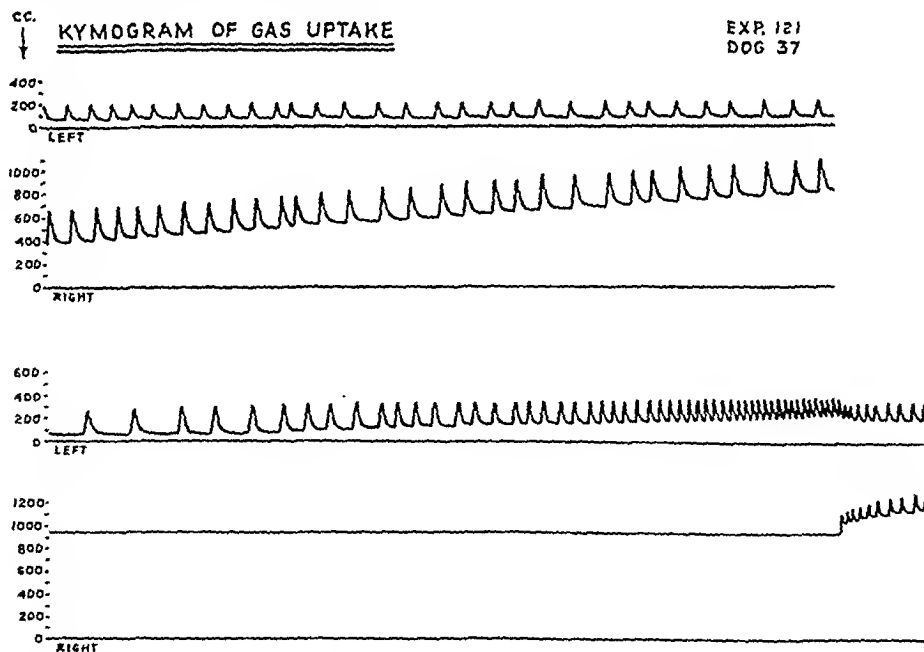


Fig. 4. A. UPPER PAIR OF GRAPHS: gas transfer with both lungs breathing oxygen. B. Lower pair of graphs: gas absorption of left lung, while the right lung is cut off. At the end of this run oxygen is again admitted to the right lung. Five-minute runs. Kymograph at fast speed.

III. Both lungs breathing oxygen.

IV. Left lung breathing oxygen, right lung blocked off.

The conditions of the experiment can best be analyzed by reference to a specific example (figs. 3 and 4):

I. With cannula in place, the left lung is connected to a spirometer containing a measured volume of room air, between 750 and 1,000 cc. The right lung breathes from the room. The systemic arterial blood arriving in the left lung via the bronchial arteries has a relatively low oxygen content (15.3 vol. %) as sampled at the femoral artery. The range of 10 determinations in 6 dogs was 8.9 to 17.1 volumes per cent, averaging 13.8 volumes per cent. The mean oxygen saturation in 7 determinations was 62.8 per cent. The low arterial oxygen content under these conditions is thought to be associated with respiratory depression and hydremia (5) and possibly with foci of pulmonary atelectasis resulting from the anesthesia together with the supine position of the animal. A considerable decrease in the gas volume of the spirometer takes place during the 20 minutes of the experiment. This decrease is due in part to oxygen consumption, but also in part to the transfer of nitrogen from the spirometer as its tension rises².

II. When the air in the left spirometer is replaced with oxygen, respirations become slower and deeper. At the same time there is a more rapid absorption of oxygen (14.8 cc/min.) commensurate with a steeper oxygen tension gradient, and the arterial blood oxygen content in the present instance rises to 16.9 volumes per cent. The mean oxygen content rose 1.9 volumes per cent and the mean O₂ saturation from 62.8 to 81.6 per cent in the same animal reported in *phase I* of the experiment. Under these circumstances the decrease in the gas content of the spirometer is due solely to absorption of oxygen. Actually more oxygen is absorbed than is indicated by loss in volume, since diffusion of nitrogen now takes place from pulmonary capillaries into the spirometer in amounts usually ranging between 100 and 200 cc. in 20 minutes. (The average of 20 determinations was 156 cc/20 min.)

III. With the left lung still breathing oxygen, the right lung is now supplied with almost pure oxygen from its spirometer. Under these circumstances, the absorption from the left spirometer falls strikingly. The respiratory rate usually decreases, and the depth of respirations increases. The blood now has an even greater oxygen content than can be accounted for by the hemoglobin content as determined by the carbon monoxide capacity. In the present instance the oxygen content during *phase III* was 19.9 volumes per cent and the carbon monoxide capacity was 19.5 volumes per cent. In 42 determinations the oxygen saturation in *phase III* was less than 100 per cent in only 6 instances. This high oxygen content is to be expected from the absorption coefficient of the plasma. The plasma alone will dissolve as much as 2 volumes per cent of oxygen when in equilibrium with tensions of oxygen in excess of 700 mm. Hg (16).

The small uptake of oxygen when the bronchial arterial blood reaches the alveoli of the left lung results from the fact that it is already practically in equilibrium with the alveolar tension, having been exposed to an equivalent tension on the right side.

² The composition of the gases at the end of 20 minutes was not determined in this particular experiment, but in another experiment under similar conditions (exper. 123; dog 9) the analysis showed 13.1 per cent oxygen and 86.9 per cent nitrogen at the end of this period. The total volume of gases absorbed by the left lung during the 20 minutes was 230 cc., but referring to the composition of the 1195 cc. of gases in the spirometer at the beginning of the experiment, namely oxygen 20.9 per cent, carbon dioxide 0.1 per cent, and nitrogen 79.0 per cent, it is apparent that only 124 cc. of oxygen had been absorbed while 105 cc. of nitrogen were being absorbed at the same time. Thus 53.9 per cent of the gas absorption calculated from the spirometer record represented oxygen. These calculations take into account the dead space in the spirometer system.

Some reduction in saturation occurs from admixture of desaturated venous blood which enters the pulmonary veins, or the left side of the heart via Thebesian channels. We have repeatedly observed, in man, sizeable branches of the pulmonary vein which drain the posterior mediastinum. Some desaturated blood must also come from the walls of the bronchi and supporting structures of the lung.

IV. In the last section of the experiment, after the control interval of 5 minutes with both sides breathing oxygen, and while the kymograph is moving at fast speed (fig. 4A), the oxygen supply to the right lung is cut off for 5 minutes by clamping the rubber tubing inlet (fig. 4B). The oxygen content of the blood in the bronchial artery falls rapidly, approaching that of the mixed venous blood whose oxygen content is rapidly diminishing. The conditions of maximal absorption of oxygen by the blood actually perfusing the alveolar capillaries of the left lung now obtain. Inspection of the kymogram (fig. 2) reveals that the respirations at once become deeper and that their rate increases, particularly toward the end of the 5-minute interval. It is also obvious that the tracing of the gas absorption now is a curve with an increasingly steep slope, while the tracings during the previous phases of the procedure were linear. It will be noted that during the previous phase of the procedure the animals have had time to approach nitrogen equilibrium with the gases in the spirometer. Thus, during the 5 minutes of *phase IV*, under the influence of the steep gradient between the high tension of O_2 in the left spirometer and the deoxygenated blood, the absorption curve represents uptake of oxygen unmodified by significant nitrogen transfer.

The left lung becomes overexpanded as the right becomes increasingly atelectatic as indicated by the drop in the resting level of the left spirometer when the right lung is allowed to reexpand. Obviously the measurement of the oxygen absorbed must be referred to the resting level of the left spirometer after reexpansion of the right lung. Reexpansion occurs within the first few breaths after unclamping the right inlet tube. The right lung, however, may not reexpand quite to its former volume, introducing an error into the calculation of oxygen absorption by the left lung. In the present instance the oxygen absorbed from the left spirometer was 28.6 cc/minute.

Towards the end of the 5-minute interval the animals as a rule become severely dyspneic, or even apneic in the case of those with the more recent ligations. The bronchial circulation of the left lung is insufficient to support life for more than a few minutes, even 21 months after ligation of the pulmonary artery.

Oxygen Absorption of the Lung in Relation to Time After Ligation of the Pulmonary Artery. When the oxygen supply of the intact right lung is interrupted (as in *phase IV* of the experiment just described), the oxygen absorption of the left serves as a rough measure of the blood flowing through that lung. Analysis of the data, table 1 and figure 5, shows an upward trend as the time after ligation of the left pulmonary artery increases. Sixty-seven observations on 10 dogs are recorded. The trend line shown in figure 1 was established by a statistical analysis and is found to be highly significant (p less than .01). The slope of this line is indicated by the formula $Y = 17.16 + 1.69 X$.

Further consideration of the factors in the experiment is necessary for a proper interpretation of the data. The factor of atelectasis of the right lung during the ob-

TABLE 1. ARTERIAL BLOOD O₂ LEVELS, AND GAS UPTAKE AND FLOW IN BRONCHIAL ARTERY IN LUNG WITH LIGATED PULMONARY ARTERY

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TABLE I—Continued

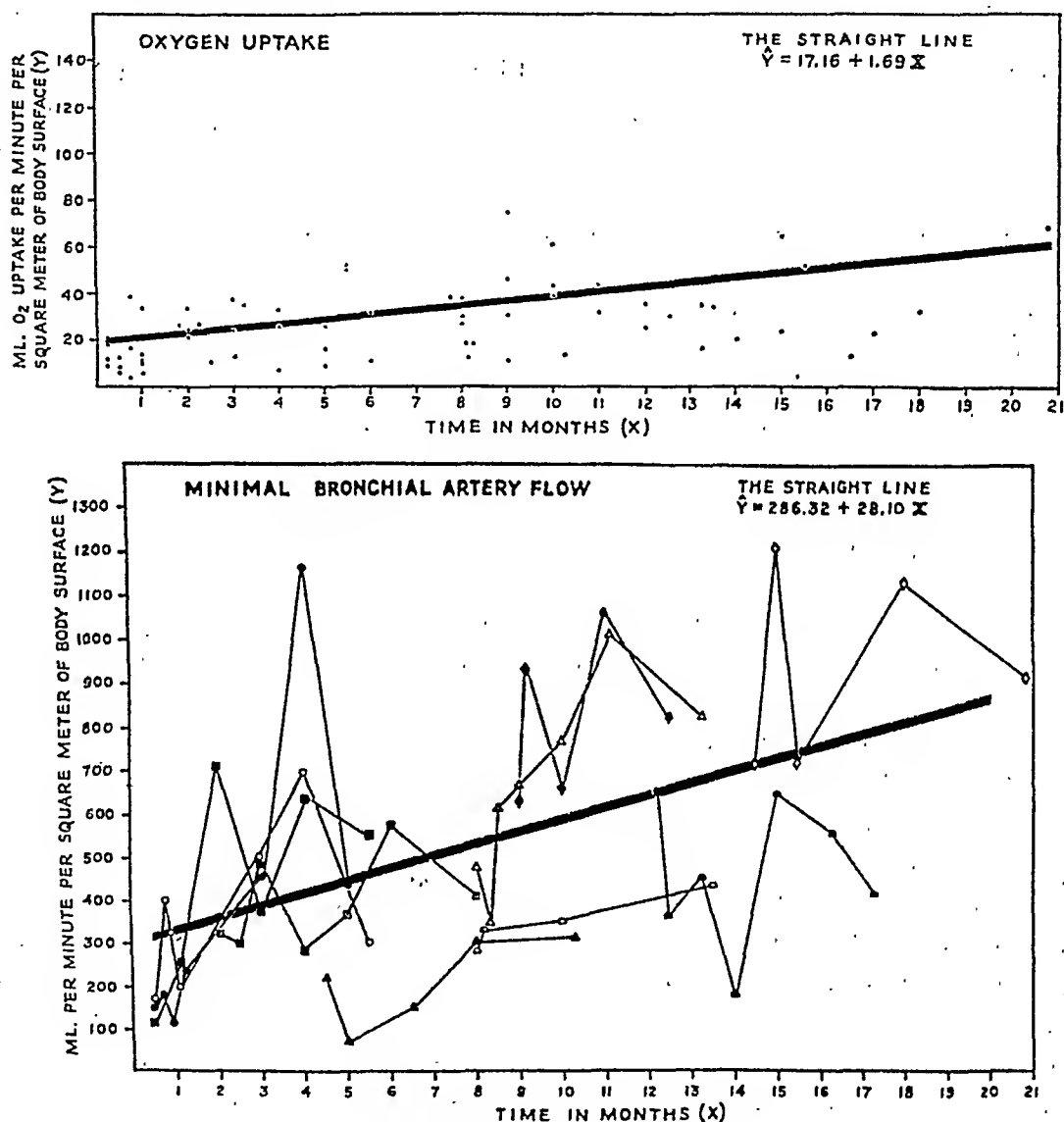
DOG NO.	SURF. AREA	MONTHS POST-OP.	Phase II				Phase III	Phase IV	MINIMAL FLOW	ESTIMATED FLOW
			GAS UPTAKE	O ₂ AB-SORBED	N ₂ TRANS-FERRED	BLOOD O ₂	BLOOD O ₂	GAS UPTAKE		
									cc/M ₂ /min.	cc/M ₂ /min.
50	.72	0.8						3.1		
	.81	4.5	6.6			13.3	16.6		223	
	.74	5.0	2.4			14.4	17.4	6.9	73	
	.96	6.5	12.5			9.4	17.8		154	
	.93	8.1	6.9	13.4	6.5	13.4	15.9		301	582
	.97	10.3	10.2	15.9	5.7	14.3	17.7	14.2	313	485
52	.64	0.3						6.1		
	.64	0.5						5.1		
	.62	0.8						10.2		
	.64	2.0	3.6			14.2	15.9	13.8	331	
	.62	2.5	2.6			14.5	15.9	6.5	300	
	.64	3.0	3.9			16.4	17.6	15.8	503	
	.74	4.0	4.3			17.8	19.8	24.8	289	
	.72	5.0	5.8			17.5	19.7	11.8	367	1010
	.74	6.0	6.8	16.1	9.3	16.7	18.3	8.5	574	
	.76	8.0	7.3	15.0	7.7	16.6	18.9	29.3	414	845
55	.62	0.5	1.8			15.1	16.9	4.0	161	
	.61	0.8	2.4			15.9	18.1		179	
	.62	1.0	2.9			14.3	18.4	7.3	115	
	.62	1.0	4.7			14.8	18.0	3.7	237	
	.63	3.0	14.2			13.6	18.5	34.6	460	
	.63	4.0	9.2	17.5	8.3	18.5	19.8		1171	2233
	.61	5.0	5.1	16.4	11.3	17.8	19.8	16.2	430	1370
56	.62	0.5	8.9			13.1	21.4		173	
	.62	0.8	8.7			17.9	21.3	27.6	413	
	.63	1.0	12.7			14.2	20.3		332	
	.64	1.1	5.5			14.1	18.4	21.9	197	
	.75	3.0	14.7			16.4	20.4	28.4	491	
	.75	4.0	12.5	22.0	9.5	17.0	19.4	25.6	695	1223
	.77	5.5	6.2	11.9	5.7	20.0	22.7	39.0	299	740
57	.58	0.5	1.4			15.6	17.6	7.1	119	
	.59	1.1	5.3			13.1	16.6	6.9	256	
	.61	2.0	2.6			17.1	17.7	15.0	710	
	.59	3.0	4.9			14.9	17.1	7.7	378	
	.59	4.0	9.9	20.6	10.7	14.7	17.3	15.5	634	1317
	.61	5.5	6.8	12.8	6.0	16.0	18.0	31.7	544	1023

Phase II: Right lung breathes room air, left lung O₂ from spirometer. Phase III: Both lungs breathe O₂ from spirometers. Phase IV: Right lung cut off, left lung breathes O₂ from spirometer.

Blood O₂ (in femoral artery) given in vol.%. Gas uptake given in cc./min.

In phase II gas uptake based on 20-minute test, except experiment 172 (based on 30 min.) and experiment 142 (based on 7 min.). In phase III, gas uptake based on 5- or 6-minute run.

struction of its airway has already been mentioned as being in part responsible for the rising slope of the oxygen absorption line during *phase IV* (fig. 4B). When the airway of the right lung is reestablished this lung tends to reexpand, but its reexpansion may not at once reach pre-occlusive levels. Thus the estimates of oxygen absorption may be somewhat too high. The slope of the trend line, however, should not be disturbed by the factor of incomplete reexpansion of the right lung since this should be roughly the same from one determination to another.



Figs. 5 (upper) and 6 (lower)

A study of table 1 reveals that the oxygen absorption per minute during *phase II* may in some instances exceed that of *phase IV*. This suggests that there may be a fall in cardiac output which offsets the effect of the steeper oxygen gradient.

Calculation of the Effective Bronchial Arterial Blood Flow. By 'effective' flow is meant that flow taking part in the absorption of oxygen, i.e., the blood that reaches the capillaries of efficiently ventilated alveoli. Bruner and Schmidt (17) present evidence that in the dog, only about two-thirds of the blood in the 'bronchial

arteries' actually enters the lung. Following ligation of the pulmonary artery there is added to the blood arriving via the main bronchial arteries blood from collateral channels. As demonstrated in anatomical studies (6), this includes branches from the enlarged esophageal and pericardiophrenic vessels. For convenience in discussion, blood distributed to the alveoli from these channels is also included under 'Effective B.A.F.'.

The Effective B.A.F. can be calculated from the following application of Fick's principle: Effective B.A.F. = $\frac{a}{c - b} \times 100$, where a is the O_2 absorbed in ml/min.

in the left lung, c is the O_2 content of blood leaving the alveoli of the left lung, and b the O_2 content of the blood in the bronchial artery in volumes per cent. If it is assumed that b is the same as the O_2 content of the blood in the femoral artery from which samples can conveniently be obtained, then the only undetermined element that remains is c . This may be assumed to be the same as the O_2 content of the systemic arterial blood when both lungs are breathing O_2 , a value obtained by analysis in the immediately subsequent *phase III* of the experiment as has been described.

Minimal Effective Flow. The minimal effective flow can be calculated from data obtained during *phase II* of the experiment. By 'minimal flow' is meant that the effective blood flow is at least of the stated volume. During *phase II* the measured rise in the curve (fall in spirometer volume) represents the excess of oxygen absorbed over nitrogen transferred into the spirometer, as has been stated. Under the conditions of the experiment, the volume of oxygen absorbed always exceeds that of the nitrogen delivered. This results from the fact that at any particular blood circulation rate, the absorption gradient for oxygen, which is determined largely by the unsaturated hemoglobin entering the capillaries of the alveoli, is greater than the secretion gradient for nitrogen which results from the difference in tensions of the nitrogen dissolved in the blood and that in the spirometer. As blood flow increases this disproportion is further displaced in favor of oxygen absorption.

If the value ' a ' for the formula is obtained from *phase II*, it therefore represents the minimal value for the rate of oxygen absorption. Thus a 'minimal effective bronchial arterial flow' can be calculated. The data obtained from 60 observations on 10 dogs are plotted against time in figure 6. The successive observations on a single animal are connected by lines. Statistics obtained by summing results over all 10 dogs were used in the calculation of a best-fitting linear equation representing the regression of blood-flow on time. Analysis showed that linear regression accounted for a very significant percentage of the total variation ($p < .01$).

Effective Bronchial Arterial Flow. To obtain an estimate of the true 'effective bronchial arterial flow' the oxygen actually absorbed was determined by analysis of the gases in the spirometer at the end of *phase II*. The flow was calculated from the formula given above. Nineteen observations were made on 10 dogs. These are summarized in table 1. In 16 of the observations the flow exceeded 700 cc/M²/min., in 12 it was in excess of 1 l/M²/min., and in 4 it was greater than 1500 cc/M²/min. The highest estimated flow was 2233 cc/M²/min.

DISCUSSION

The magnitude of the flows observed is surprising, especially in view of Bruner and Schmidt's (17) studies of normal dogs with the bubble flow meter. They found that the flows did not exceed approximately 30 cc. per minute even under maximal vasodilation induced by drugs. The large variations in flow observed in the present determinations probably depend in part on the depth of the sodium pentobarbital anesthesia. It is possible also, in some experiments, that, despite precautions, the tip of the bronchspirometric cannula may have been beyond the orifice of the left upper lobe bronchus. In Bruner and Schmidt's experiments there was evidence of a striking rhythmic vasomotor activity in the bronchial vessels of the relatively normal lung. Whether this may persist under the conditions of the present experiment is unknown. The mechanism of the remarkable expansion of the bronchial circulation that takes place after ligation of the pulmonary artery is not clear. Most of the increase in flow occurs within the first few weeks. Possibly the responsible factor is the fall in pressure within the capillary beds held in common by the two circulations when the flow in the pulmonary artery stops. Miller (12) has demonstrated these capillary beds to lie within the walls of the respiratory bronchioles. The direction of flow in these elongated fine meshed capillaries may vary depending on the relative pressures in the terminals of the bronchial and pulmonary arteries (13). Under the present conditions blood from the bronchial vessels tends to perfuse the capillaries of the alveoli where transfer of gases is efficiently accomplished.

Anatomical studies demonstrate also the opening up of easily visible pre-capillary anastomoses between the bronchial and pulmonary arteries. The reasons for their development are not clear.

Once the bronchial arteries have increased in size, by whatever mechanism, it is not difficult to understand why they should carry a large flow: the presumably low peripheral resistance in the lungs together with a high pressure at the source. Huge flows through enlarged bronchial arterial channels have also been observed in cases of congenital pulmonic stenosis (9). It is obvious, moreover, despite the absence of exact data, that the hydrostatic pressure in the capillaries of the lung supplied only by an expanded bronchial arterial circulation does not exceed the colloid osmotic pressure of the blood—else the alveoli would filter like the glomeruli of the kidney. A part of the impact of systole in the bronchial arteries is cushioned by their large communications peripherally with the persistently patent sac-like branches of the pulmonary artery.

SUMMARY

In the dog, a lung with a ligated pulmonary artery can maintain a respiratory function. The capacity of such a lung to absorb oxygen gradually increases over a period of months. When oxygen rather than air is supplied through a bronchspirometric cannula, while the intact lung continues to breathe air, the oxygen content of the arterial blood is found to rise. The effective flow in the bronchial arteries of such a lung increases with time after ligation, as measured by bronchspirometry and an application of the Fick principle. The increase in circulation is in step with the expansion of the vascular bed demonstrated in anatomical studies by the vinylite

method as reported elsewhere. After the fourth month with the animal under sodium pentobarbital anesthesia, the flow usually exceeds $1\text{ l/M}^2/\text{min}$. This flow is largely a burden on the left heart whose output becomes roughly one-third greater than that of the right. A similar situation obtains in human disease, such as bronchiectasis or congenital pulmonic stenosis, where there is an extensive collateral circulation to the lungs.

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MYENTERIC REFLEX

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THE question of an enteric nervous mechanism capable of independent reflex activity has been under investigation since Nothnagel (1) and Luederitz (2) first noted that stimulation of the gut usually produces a contraction orad but not caudad. Luederitz also observed that this response could still be obtained after denervation and, therefore, considered it to be due to a local reflex which later was called the myenteric reflex by Cannon (3).

Although confirmed by Bayliss and Starling (4), partly also by Magnus (5) and Cannon (3, 6) recent investigators have cast doubt on the significance and even the existence of this reflex. In extensive studies on the rabbit, Alvarez (7, 8) could not find it. Ganter (9) and Rodin (10) and others failed to observe it in human subjects (cf. 7). Raiford and Mulinos (11, 12), on the other hand, demonstrated a reflex similar to the myenteric reflex in transplanted segments of the colon. They found that stroking the mucosa produced a contraction of the circular muscles above and of the longitudinal muscles below the stimulated region. Hukuhara (13) noted that pinching caused a stronger muscular response orad than caudad.

This question was reinvestigated using a variety of methods and preparations. The results obtained confirm the existence of an enteric reflex which involves a synaptic mechanism. It can be elicited by mild mechanical stimulation such as stroking the mucosa, or longitudinal stretching. These stimuli produced, on the oral side of the stimulated region, an augmentation of rhythmic activity or a prolonged powerful contraction with temporary cessation of rhythmic movements. This response does not progress along the intestine.

As will be shown below, the reflex response varies considerably under different conditions. The only constant characteristic of the reflex is its polarity. Therefore, in the following, all responses which show this property will be called myenteric reflex. The question whether the reflex also involves a descending inhibition, as claimed by some authors, will be discussed later.

In view of the simplicity of the experiments described below it may seem surprising that so much difficulty has been encountered in demonstrating the essential facts concerning enteric reflexes. The variability in the responses obtained is partly due to the use of abnormal, crude stimuli by many investigators. Confusion has also been introduced by the term peristalsis. The myenteric reflex comes into play in the transport of a bolus. It is erroneous, however, to assume that every wave of contraction in smooth muscle is caused by the reflex. Simple waves of contraction, such as those of the stomach and ureter, are due to muscular conduction, whereas peristalsis caused by a bolus is a much more complex phenomenon (14). It is unfortunate that the term peristalsis has been used to designate these two vastly different types of activity. The term reflex peristalsis, therefore, has been proposed for the peristaltic waves due to the myenteric reflex.

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METHODS

Anesthetized dogs (morphine, 3 mg/kg. subcutaneously and sodium barbital, 200 mg/kg. intraperitoneally), and rabbits (sodium pento-barbital intravenously) were used. Short lengths of intestine were exposed and covered with dry cotton except during observation. The room was kept at a temperature of 27° or higher and was often humidified. Electric stimuli were applied through platinum hand electrodes. Repetitive stimuli at a frequency of 20 to 50 shocks per second, supplied by a thyrotron stimulator discharging over a transformer, were used.

Action potentials were recorded by the technique described previously (22). Monophasic potentials were obtained by placing one lead on a small mass of tissue firmly tied off by a silk thread, the other lead on another region at the same level (16). The resulting injury diminished spontaneous motility in this region but did not prevent the passage of a peristaltic wave.

RESULTS

Normal Stimulus. In the dog's intestine, lateral distension, produced by spreading a hemostat introduced into the lumen, increases the strength of the rhythmic contractions on the oral side without any noticeable effect caudally. Usually, however, a stronger response is elicited by stroking the mucosa with a blunt instrument introduced through a small opening. Stroking the serous surface on the other hand, is ineffective. It may be concluded, therefore, that specific receptors for the myenteric reflex responding to mild mechanical stimulation are present in the mucosa.

The response to stroking probably is important for the initiation of reflex peristalsis by the introduction of a bolus. That distension is not necessary is indicated by the fact that a bolus so small that it does not distend the wall of the relaxed intestine can elicit peristalsis. Furthermore, stroking the mucosa in the region of a bolus often starts a peristaltic wave under conditions where the responsiveness of the intestine is low.

Curiously enough, in the rabbit's small intestine, stroking the mucosa never elicits a response. Lateral distension is effective only in rather sensitive preparations. However, longitudinal distension, gently performed by stretching a short piece of gut with the fingers, produces strong ascending contractions of the circular muscles without any effect on the aboral side (fig. 1). In the upper part of the small intestine, the response may extend orad for 5 to 10 centimeters and may last for half a minute, whereas farther down in the intestine often only a brief contraction or none at all is produced by the stimulus. The contractions are often rhythmic, differing from spontaneous activity only by the participation of the circular muscles, or they are single contractions lasting for as long as 20 seconds.

The differences in the character of the effective stimulus in the preparations studied are probably related to the different consistency of the intestinal contents under normal conditions and may be considered an adaptation to the prevailing type of mechanical stimulus.

That smooth muscle can also be stimulated directly by stretching has been shown by a study of action potentials in the ureter (17), but, since the response so elicited

has no polarity and consists of a single all-or-none conducted impulse, it evidently has no relation to the myenteric reflex.

Effect of Electric Stimulation. Luederitz (2) reported that electric stimulation produced chiefly an ascending contraction in the rabbit's intestine, but Alvarez *et al.*

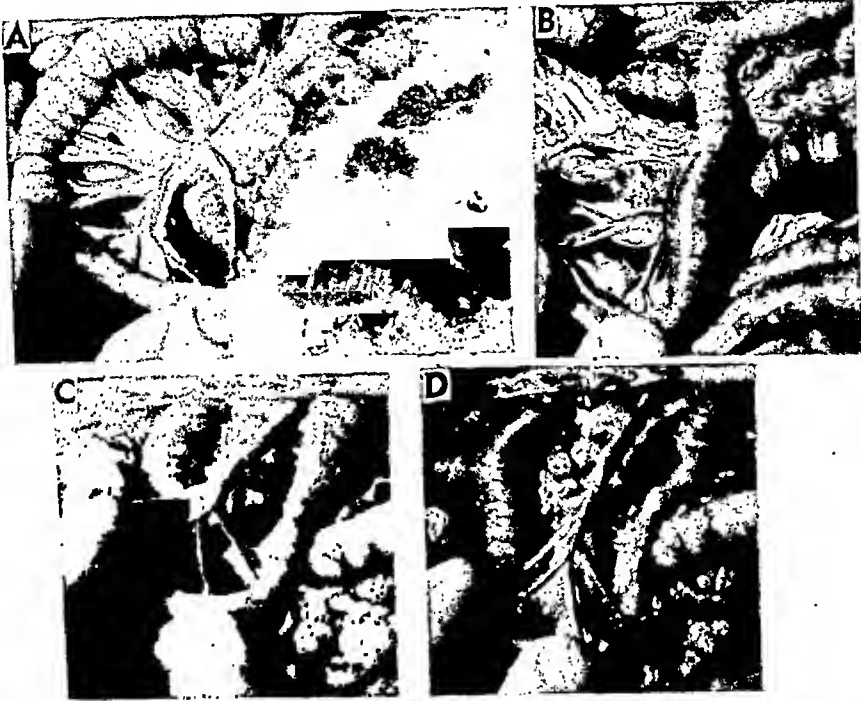


Fig. 1

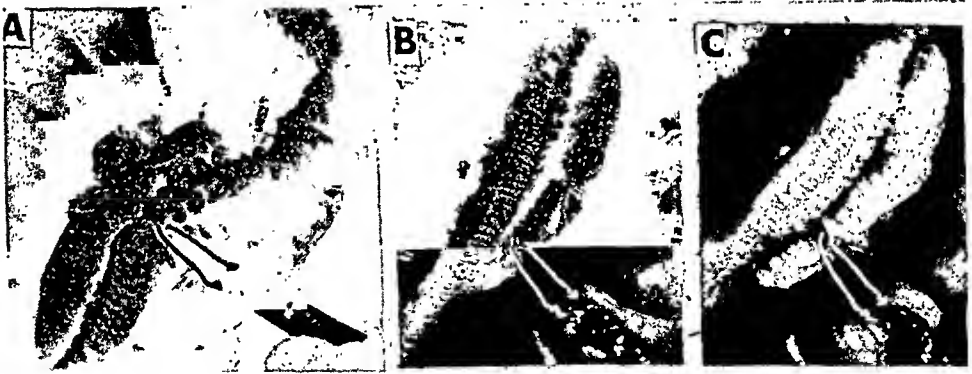


Fig. 2

(7, 18) found the responses very variable, spreading, on the average of many observations, slightly farther caudad than orad.

In my own studies, the effects of electric stimulation varied in different parts of the intestine and under different conditions, but were reproducible. Strict polarity of the response was always observed in the proximal colon of the rabbit. Electric stimulation of a small region in this part of the intestine increased the activity of the haustra, increased the tonus of the circular muscles and often caused rhythmic contractions of the longitudinal muscles orally, never on the other side (fig. 2A).

In most parts of the rabbit's small intestine, electric stimulation can produce ascending and descending contractions. However, stimuli not more than 50 per cent above threshold always give strictly polar responses in all parts of the intestine (fig. 2B). Such weak stimuli are effective only after they have been applied for 5 to 10 seconds, showing the importance of summation in this reflex.

Weak electric and mechanical stimulation produces strong ascending and descending contractions during the period of hyperexcitability following the cessation of circulation. Similar nonpolar responses which are produced by stretching and are abolished by low concentrations of nicotine were obtained in isolated intestine by Fleisch (19).

Whether the descending response to electric stimulation, which normally has a higher threshold than the ascending contraction, is a part of the myenteric reflex or

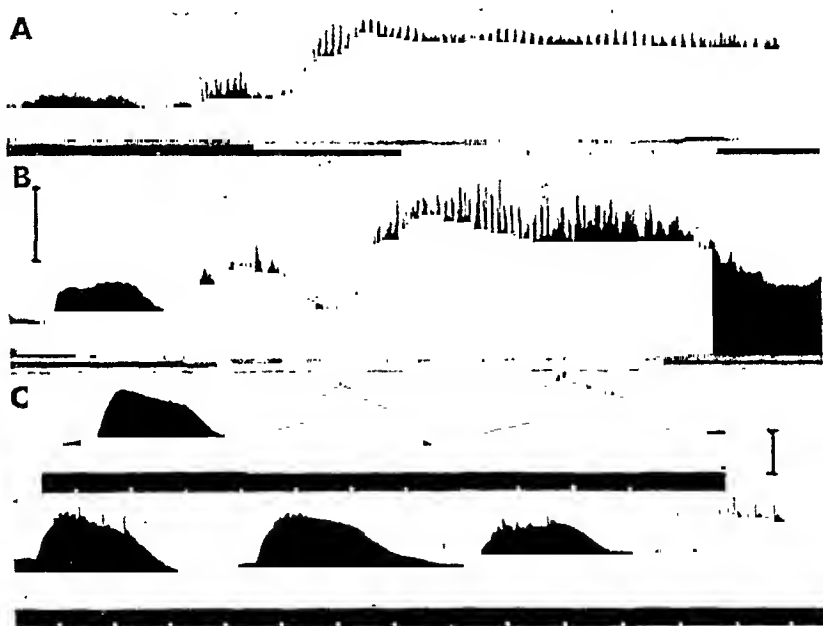


Fig. 3

belongs to some other reflex mechanism cannot be decided at present. It is possible that the polarity of the reflex is only relative, stimulation causing a quantitatively different effect orally and aborally. These considerations are important for the question of anti-peristalsis which will be discussed later.

Muscular Response. The character of the response varies in different preparations and depends on the strength of stimulation. In the dog's small intestine, stroking always increases the strength of the spontaneous contractions without changing their frequency. This agrees with the observations on peristalsis which, likewise, usually is rhythmic (14).

Action potentials show a characteristic difference between duodenum and ileum. In the former, spikes are very prominent and have a high frequency, whereas, in the latter, the slow potentials (20-22) are very large. In the ileum of the rabbit the reflex responses always remain rhythmic, whereas in the duodenum the response to stretching may be sustained for 8 to 10 seconds (fig. 3).

Bayliss and Starling (4) and others (3, 13) observed that pinching the intestine usually produces a descending inhibition and that a wave of inhibition precedes peristaltic waves. Auer and Krueger (23) presented evidence for a wave of inhibition preceding both peristalsis and anti-peristalsis in the colon of the rabbit. They based their conclusion mainly on the observation that, if two peristaltic waves approach each other, one or the other stops. This, however, is not a constant phenomenon, because Alvarez (7) and I have observed strong and opposing contractions on both sides of a bolus. In my experiments, the colon usually was at complete rest, except for the region oral to the bolus, as indicated by the absence of action potentials. In the dog's small intestine, electric activity and motility, as far as it could be observed visually, were not diminished ahead of a peristaltic wave.

One may conclude from the literature on the subject that descending inhibition sometimes is associated with the myenteric reflex but that it is not a constant phenomenon. It is doubtful, therefore, whether it should be considered as a part of the myenteric reflex.

The observations by Youmans, Meek and Herrin (24) perhaps have an important bearing on this problem. These authors found that distension can cause inhibition of the denervated intestine extending beyond the stimulated region. Although this effect does not seem to have any polarity, it might, under appropriate conditions, mask a descending excitatory effect or cause inhibition without suppressing the strong ascending contractions. Bayliss and Starling themselves report that pinching sometimes caused ascending as well as descending inhibition which agrees with the conclusion that inhibition is not polar and is not an essential part of the myenteric reflex.

Coordination of Circular and Longitudinal Muscles. In reflex peristalsis the contractions on the caudal side of a bolus usually involve only the longitudinal muscles as indicated by the fact that they do not narrow the lumen, whereas the ascending contractions constrict the intestine and cause elongation, showing that the contraction of the circular muscles is predominating. In agreement with these observations Raiford and Mulinos (11, 12) found that stimulation of the colon of the dog caused contraction of the longitudinal muscles caudad, of the circular muscles oral. The studies of Auer and Krueger (23) on the colon of the rabbit also are essentially in agreement with these conclusions.

To explain the observations just described it is not necessary to assume a separate nervous control of the two muscle layers. Quite generally, during weak contractions, chiefly the longitudinal muscles are active, producing a shortening of the intestine, whereas strong contractions produce elongation, evidently due to the greater strength of the circular muscles. The relation between the two muscle layers, therefore, may be explained on the assumption that the circular muscles have a higher threshold than the longitudinal muscles. It is possible, also, that the nervous elements responsible for the myenteric reflex make connection preferentially with the circular muscles.

Mechanism of Conduction. The character of the response suggests that a nervous mechanism conducting chiefly in an oral direction is involved. That muscular conduction cannot account for the effect of stimulation is confirmed by the obser-

vation that the contractions often originate at some distance from the region stimulated. In the terminal ileum of the rabbit, strong contractions often first appear 2 to 3 mm. oral to the stimulating electrodes and only later spread to the electrodes. In the duodenum, the contractions produced by longitudinal stretching often start 5 to 10 cm. above the stimulated region (fig. 1B).

The principal question is the explanation of the polarity of the response. Raiford and Mulinos have proposed that nerve fibers are running from the mucosa oral directly to the muscle fibers, thus representing an axon reflex arc. Since the effect of local stimulation extends for several centimeters, these nerve fibers must be assumed to be very long. If they had terminations on muscle fibers over their entire length, electric stimulation would be expected to produce a response oral as well as caudad. As shown above, however, the responses generally spread caudad less than a millimeter. Therefore, on the assumption of an axon reflex, it must be assumed, furthermore, that each fiber has motor endings only at one certain distance from the sensory endings.

The polarity can be explained more readily by assuming a synaptic mechanism conducting preferentially in an oral direction. This possibility can be tested by the action of drugs. It is well-known that peristalsis of the intestine, in contrast to ordinary rhythmic activity, is abolished by the intravenous injection of small quantities of nicotine, an observation which has been considered as evidence for a synaptic mechanism (4, 25). However, the effect of the drug cannot be interpreted with certainty in such experiments because the possible effects of the drug on receptors, synapses and the muscle has not been evaluated.

In the present work, drugs were applied by placing narrow strips of cotton soaked in the solutions all around the intestine. Rabbits were used. Nicotine sulfate or pure nicotine in high concentrations, 1:1000 to 1:5000, produced strong ascending and descending contractions of the circular muscles. Following this response, which passed off in one to two minutes, the response to local stimulation did not spread beyond the nicotinized region (fig. 1, 2). In concentrations of 1:10,000 to 1:20,000, nicotine usually blocked the spread of the response without first producing a contraction. All of these effects were completely reversible.

Atropine in concentrations as low as 1:10,000 and tetraethylammonium chloride as low as 1:1,500 blocked conduction like nicotine. It was unexpected that adrenalin (1:50,000 or higher) also blocked conduction in many animals. However, the minimal effective concentration of these drugs, while constant in one animal, varied considerably in different animals. In the least sensitive rabbits, 14 of a group of 26 animals, adrenalin and tetraethylammonium chloride did not block completely at concentrations which were so high (1:10,000 and 1:500 resp.) that other effects of the drugs (vascular effects and local contractions) became prominent. This group was also less sensitive to the action of nicotine and atropine.

Acetylcholine at the lowest effective concentrations produced a weak local contraction, probably due to direct stimulation of the muscle. At slightly higher concentrations (about 1:2000 for the duodenum, 1:10,000 for the lower ileum) only an ascending contraction occurred. Still higher concentrations also gave a descending

response. These observations indicate that acetylcholine stimulates the muscle directly and indirectly through a nervous plexus.

Since none of the drugs mentioned, even in concentrations much higher than those used here, stimulate or block nerve fibers, it may be concluded that the reflex responses involve a synaptic mechanism. Furthermore, because the block is sharply limited and because generally the response to stimulation does not spread caudad noticeably, the neurons involved in the reflex must be assumed to be short.

Summation and Fatigue. The myenteric reflex has some of the characteristics of reflexes involving the central nervous system. Brief stimulation generally is ineffective. The weaker the stimuli the longer they have to be applied before a response appears. The importance of summation is strikingly illustrated by the responses to weak electric stimuli described above.

The reflex, furthermore, is subject to rapid and long-lasting fatigue. On continuous stimulation, the reflex generally subsides within 1 minute, often within 10 seconds. Following a strong response, stimulation is entirely ineffective for half a minute or longer.

The importance of summation and fatigue is also evident from observations on peristalsis (14). The introduction of a bolus into the small intestine of the dog usually does not initiate a peristaltic wave immediately. It first produces a gradual increase of rhythmic activity on the oral side. A peristaltic wave begins only when the contractions are strong enough to propel the bolus.

COMMENTS

The observations described here show that the myenteric reflex involves an enteric nervous plexus which conducts decrementally, chiefly in an oral direction. It consists of short neurons connected by synapses. Pharmacologically the synapses are similar to those of the autonomic nervous system, being blocked by nicotine and tetraethylammonium chloride and stimulated by acetylcholine. The blocking action of atropine and adrenalin is peculiar but Marrazzi (26, 27) has demonstrated a depressing action of these drugs on sympathetic ganglia.

The relationship of the plexus responsible for the myenteric reflex to the autonomic system is uncertain. It appears improbable, however, that the plexus consists merely of post-ganglionic parasympathetic neurons because vagal stimulation never sets off as powerful a response as can be elicited by appropriate stimulation of the intestine.

The polar character of the myenteric reflex explains why the peristaltic waves of the intestine generally travel only in an aboral direction. The reflex by itself, however, does not set up peristaltic waves as shown by the fact that the reflex response to local stimulation never progresses along the intestine and that such waves cannot be produced in the empty intestine. Their propagation is due to the continuous stimulation by the contents of the organ which reinforces the muscular activity on the oral side, particularly that of the circular muscles.

This type of peristalsis should not be confused with the peristaltic waves of the stomach and ureter. The waves of contraction of these organs are analogous to single impulses in cardiac muscle. They can be elicited by single electric shocks.

They are all-or-none responses and are conducted equally well in both directions. These and other observations on the effect of electric currents and on action potentials (15, 20, 21, 22, 28) agree with the assumption that conduction is purely muscular. The fact that, in contrast with true peristaltic waves, conduction is not blocked by high concentrations of cocaine and nicotine (28, 29) leads to the same conclusion.

Because the action potentials of the rhythmic contractions of the intestine are essentially like those of the ureter and the stomach, it is evident that the individual contractions in all of these organs involve muscular conduction. The chief difference lies in the fact that in the intestine single contractions are generally conducted only for short distances, a condition which is largely due to the independent initiation of activity in many parts of the organ. The peristaltic waves of the intestine can travel long distances but, as shown above, they are composed of a series of rhythmic contractions, each one travelling only a short distance.

Some investigators have occasionally observed anti-peristalsis. It can be seen frequently in the rectum of the rabbit (23). In my own studies it was often seen in this organ, but only when the intestine was depressed, for instance after several peristaltic waves were induced in succession. Peristalsis, then, was weak. A wave often came to a standstill and a new wave started in the opposite direction. This phenomenon indicates that the polarity of the enteric nervous system is not absolute. There is, in fact, some evidence indicating that impulses are conducted in this system also in a caudal direction and that the difference in the response to stimulation on both sides is purely quantitative. It is conceivable, therefore, that under certain conditions, for instance in fatigue, the effect on the caudal side predominates, thereby producing anti-peristalsis.

Polarity has not been demonstrated in other visceral smooth muscles. It is true that in the ureter and stomach the waves of contraction normally travel in a caudal direction. For the ureter, probably also for the stomach, this observation is explained by the fact that the upper end of the organ has the highest degree of automaticity, thereby acting as the pacemaker.

SUMMARY

Local stimulation of the intestine produces responses which spread considerably beyond the stimulated area. Under certain conditions the contraction is restricted to the oral side. Such polar responses, which are designated as the myenteric reflex, may consist merely of an increase in the strength of the rhythmic contractions or they may be more prolonged contractions, chiefly of the circular muscles. The most effective stimulus for the myenteric reflex is longitudinal stretching in the small intestine of the rabbit, stroking the mucosa in the small intestine of the dog. Abnormal stimuli such as electric stimuli or pinching often produce ascending and descending contractions. However, electric stimulation always produces a strictly polar response in the proximal colon of the rabbit, and in other parts of the intestine if the stimuli are close to threshold.

Nicotine in low concentrations applied locally prevents the spread of the response from the stimulated area. Local application of acetylcholine in *minimal* concentrations produces a polar response like that caused by appropriate mechanical or elec-

trical stimulation. It is concluded that a synaptic mechanism consisting of short neurons and conducting chiefly in an oral direction is involved in the myenteric reflex.

The results are in agreement with the conclusion that the spontaneous contractions of the intestine are myogenic but are ordinarily conducted only for a short distance. The myenteric reflex increases this activity on the oral side and thereby propels the contents of the intestine. The reflex response by itself is not propagated. The peristaltic waves depend on the continuous stimulation by the contents of the intestine.

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REFLEX PERISTALSIS OF THE INTESTINE

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A SOLID object introduced into the intestine induces powerful contractions of the circular muscles which transport the object in an aboral direction. This response has been known for a long time (1-5). In the present paper it will be described in greater detail on the basis of visual observations and records of action potentials. In a subsequent paper the underlying neuromuscular mechanism will be analyzed.

MATERIAL AND METHODS

The small intestine of the dog and the descending colon of the rabbit were used. Dogs were anesthetized with morphine (3 mg/kg. subcutaneously) and sodium barbital (200 mg/kg. intraperitoneally). The use of morphine in the dog is important because under the influence of barbiturates alone motility is weak and peristaltic waves can be elicited only after long delays. Also without morphine, any manipulation of the intestine, the introduction of the bolus itself, produces a further diminution of motility, a reflex which is not disturbing if morphine is given. Neither purgation nor denervation, as recommended by Bayliss and Starling, was necessary. Rabbits were anesthetized with sodium pentobarbital, injected intravenously.

A piece of intestine about 6 cm. long was attached to a cork board by two pins, and longitudinal incisions were made at both ends. The board was rigidly clamped. For recording action potentials, the differential, non-polarizable electrodes previously described (8) were used in most experiments. Because some difficulty was experienced in preventing slipping during strong contractions, other types of electrodes were tried, but all gave essentially the same type of records. For very strong contractions it proved best to sew fine silk threads on the intestinal wall and connect them to the non-polarizable electrodes. Metallic electrodes directly in contact with the tissue were unsuitable because they produced large movement artifacts. For recording, an oscillograph or a mechanical recorder driven by a d.c. amplifier was used.

Varnished pieces of lead of different sizes were used as boluses. They were spindle-shaped, somewhat flattened on two sides so as to minimize the vertical movements of the intestinal wall during peristalsis. The experiments were carried out at a room temperature of 27° or higher. The air was often humidified. The exposed part of the intestine was covered with dry cotton except during observation.

RESULTS

According to previous descriptions a peristaltic wave is simply a strong contraction traveling in an aboral direction. It was observed, however, that the response to

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the introduction of a bolus is often rhythmic and that it varies in different species and even in different parts of the intestine in the same species. I shall first describe peristalsis in the small intestine of the dog.

After a bolus large enough to fill the lumen is introduced, the strength of the rhythmic contractions gradually increases on the oral side. Sometimes there is also some increase caudally, but always much less. The contractions usually originate around the posterior half of the bolus and travel from there orally. The activity on both sides of the bolus may be independent, but, as noted also by Hukuhara (3), waves of contraction originating on the oral side often travel over the bolus and beyond at a velocity of several centimeters per second. On the aboral side, the contractions are much weaker and do not involve the circular muscles. When the contractions on the oral side become strong enough to blanch the intestine, the bolus is pushed forward, each contraction transporting it for a short distance. The speed with which the bolus travels varies widely and increases, within limits, with the size of the bolus.

The contractions causing the transport of the bolus have the same frequency as those of the empty intestine and differ from the latter only by their greater strength. The peristalsis, therefore, does not represent a single wave of contractions, but rather an advancing front of strong rhythmic activity. The term 'peristaltic contraction,' commonly used for this form of activity, is not appropriate.

In very active preparations, particularly in the ileum, a more powerful response than that just described is often obtained. The bolus may travel at a speed as high as one centimeter per second, driven forward by a single powerful wave of contraction which sweeps over the whole piece of intestine under observation. In this type of peristalsis, a region of the intestine remains contracted for 10 to 20 seconds, and a piece of intestine 5 to 10 centimeters long is blanched at one time. There are, however, all intermediate stages between these waves of contraction and the rhythmic type of peristalsis described above. It seems unlikely, therefore, that any essential difference between these responses exists. Thus, the prolonged contractions may be considered as being due to a fusion of several contractions, a conclusion which is confirmed by the observations on action potentials described below.

The peristalsis of the descending colon of the rabbit has been studied by Langley and Magnus (7) and, in greater detail, by Auer and Krueger (1). This part of the intestine, in contrast to the small intestine, is completely relaxed for long periods, but spontaneous strong contractions of variable duration may occur. A bolus, if it is large enough to distend the intestine, readily induces peristalsis. This response may consist of a series of brief contractions, but more often a single prolonged contraction drives the bolus ahead. Observations by Auer and Krueger which have some bearing on the question of the neuromuscular mechanism of peristalsis will be discussed in a subsequent paper (8).

Action Potentials. It has been shown (6, 8) that the potentials accompanying ordinary rhythmic activity of the small intestine usually consist of a slow component, giving rise to R and T waves, and of brief spikes. The significance of this duality is not known but it was found that the strength of contraction is closely related to the magnitude and frequency of the spikes and has little relation to the size of the slow component. There are also marked and consistent differences between species and even between different regions of the intestine in the same species.

Thus, in the dog, the slow component is much more prominent in the ileum than in the duodenum (fig. 1).

The approach of a peristaltic wave has no effect on the action potentials. No evidence for a wave of inhibition preceding the peristaltic wave, as described by the older investigators (2, 7), was found. As the bolus passes below the leads the spike discharge generally increases and reaches its peak immediately oral to the bolus.

In the duodenum of the dog the action potentials at the height of peristalsis differ from those of the empty intestine only by the strong and prolonged spike discharge (fig. 1A). Although this activity may appear like a single prolonged contraction, the spikes are always grouped into distinct bursts showing that it does not differ fundamentally from the ordinary rhythmic activity of the intestine, in agreement with the conclusion already reached on the basis of other observations.

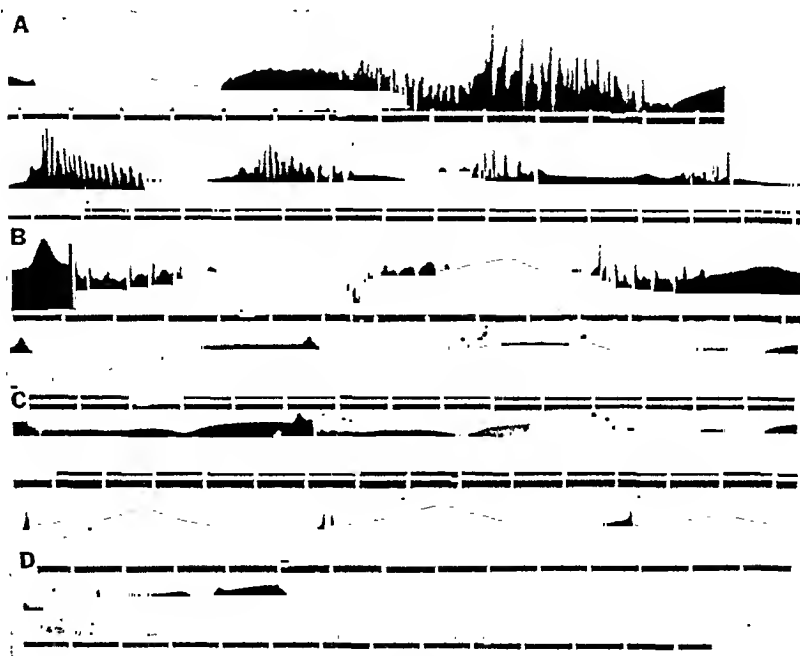


Fig. 1

In the terminal ileum, on the other hand, the spike discharge does not increase markedly during peristalsis (fig. 1B, C, D). Most surprising of all is the finding that no regular potential changes are present during the strongest contractions which cause intense blanching and last from 10 to 20 seconds (fig. 1D). Because this phenomenon was observed in dozens of cases using different types of leads, including threads sewn on the muscle, and because it never occurred in the duodenum, it seems unlikely that failure of the recording mechanism could be responsible for this unexpected result. Also various gradations from the complete absence of regular potentials to slow, but regular R and T waves, and complexes with spikes were observed. The absence of potential changes during sustained contraction shows that the muscle can remain in a continuous state of activity for rather long periods of time so that no external potential differences are present, a situation similar to that during the iso-electric phase of the electrocardiogram and of the action potentials of many visceral smooth muscles.

During the spontaneous contractions of the rectum of the rabbit, brief bursts of spikes, which seem to be superimposed on slower rhythmic potential changes, are discharged at regular intervals (fig. 2*A*). The frequency of the bursts is about one per second but increases with the strength of contraction. During the periods of rest, no potential changes can be detected, but small slow potential waves of the same frequency as the bursts often precede a discharge. The peristaltic waves are accompanied by the same type of action potentials as those of the empty organ but the frequency of the bursts may be as high as two per second at the beginning of the contraction, declining gradually later on (fig. 2*B*, *C*).

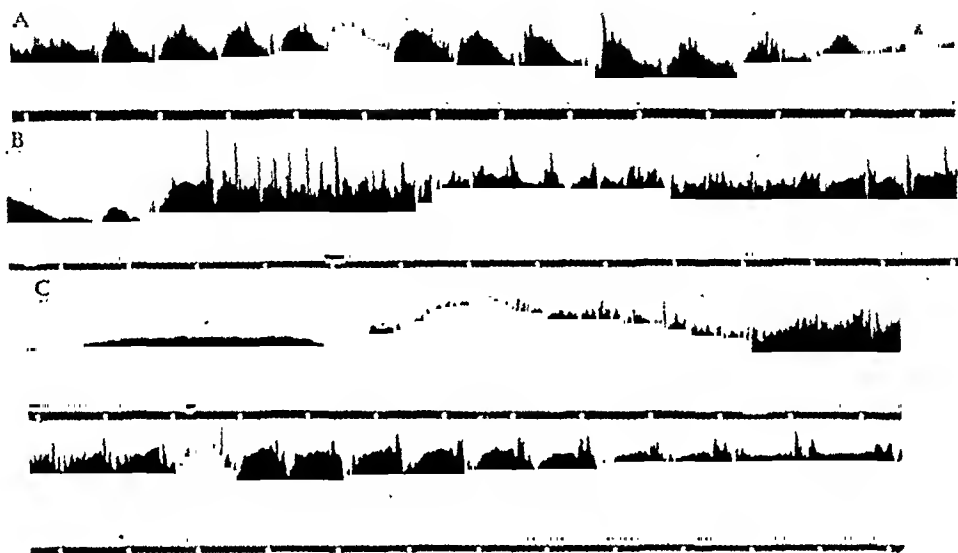


Fig. 2

COMMENTS

Since peristalsis usually is a rhythmic form of activity, it is evident that it is not comparable to the peristaltic contractions of the stomach and ureter. The latter are all or none conducted and correspond to the individual contractions which make up the peristaltic waves of the intestine. This difference is confirmed by observations on the action of drugs which will be discussed in a subsequent paper (8). The results obtained suggest that the peristaltic waves of the intestine involve a synaptic reflex mechanism whereas there is no evidence for any participation of nervous elements in the individual contractions of the ureter and stomach. The first of these types of activity, to distinguish it from simple waves of contraction, may appropriately be called reflex peristalsis because it involves a nervous mechanism.

The differences in the responses of the intestine in different species and in different parts of the digestive tract are remarkable but they do not necessarily indicate any fundamental differences in the underlying mechanisms. They also emphasize the importance of carrying out investigations on more than one type of preparation.

SUMMARY

In the duodenum of the dog the introduction of a bolus strongly increases the strength of the rhythmic contractions on the oral side, with little or no effect caudally

This activity differs from spontaneous contractions only by their greater strength and participation of the circular muscles. In strong peristalsis a single powerful and prolonged contraction sweeps over the intestine. The latter type of response is seen frequently in the rectum of the rabbit and terminal ileum of the dog, less often in the duodenum of the dog. Since all gradations between these responses can be observed, the prolonged contractions may be considered as the result of a fusion between several rhythmic contractions. This view is confirmed by a study of action potentials which differ usually from ordinary rhythmic contractions only by the greater strength of the spike discharge. However, in the ileum of the dog, no regular action potentials were observed during the strongest peristaltic waves, indicating a prolonged and continuous state of activity.



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MEASUREMENTS OF HEART OUTPUT BY ELECTROKYMOGRAPHY¹

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A SIMPLE method for accurately measuring heart output is greatly needed but up to the present time, no completely acceptable procedure has been found. The ballistocardiograph is simple and requires very little cooperation from the subject. Many, however, do not consider it reliable (1, 2, 3). Roentgenkymography (4-7) is another simple method but the subjective errors in measuring the x-ray plates may be very large—perhaps as much as 12 per cent. Electro-kymography avoids the subjective errors of measurement since its records are sharp. Furthermore, the amplitude of the movements of the heart borders may be greatly amplified. One cannot decide at the present time whether this method will prove to be as good as or better than ballistocardiography or roentgenkymography. In developing this procedure, we have compared it with ballistocardiography.

METHOD

The top of a fluoroscopic table has been removed from its base and supported by 4 vertical springs. This provides a ballistic table similar to the one described by Nickerson, Warren and Brennan (8). Movements of the table top are recorded by means of a light beam reflected from a pivoted mirror onto bromide paper. Using the same camera, the movements of the string galvanometer activated by the electrokymograph (9) are simultaneously recorded. The electrokymograph head is provided with an opening 3 cm. in diameter through which x-rays can pass to excite a fluorescent screen. A wafer grid, attached beneath this screen, eliminates the effects

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of scattered x-rays. Enclosed in the same housing and 4 cm. above the wafer grid is the 931-A photomultiplier tube (fig. 1). Beneath the top of the table, a solenoid is arranged to swing pressdwood (6 mm. thick) into and out of the x-ray beam. This is used to calibrate the movements of the heart recorded by the electrokymograph (see fig. 2). For measuring the thickness of the heart, a phantom of pressdwood 5 cm. thick is provided.

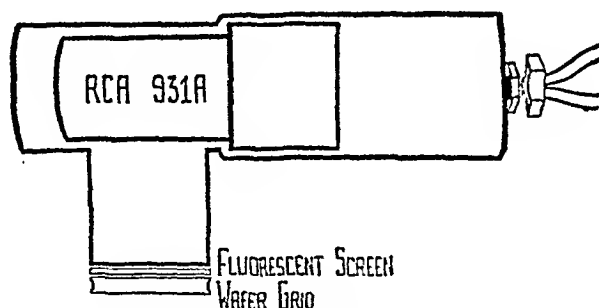


FIG. 1. ELECTROKYMOGRAPH HEAD.

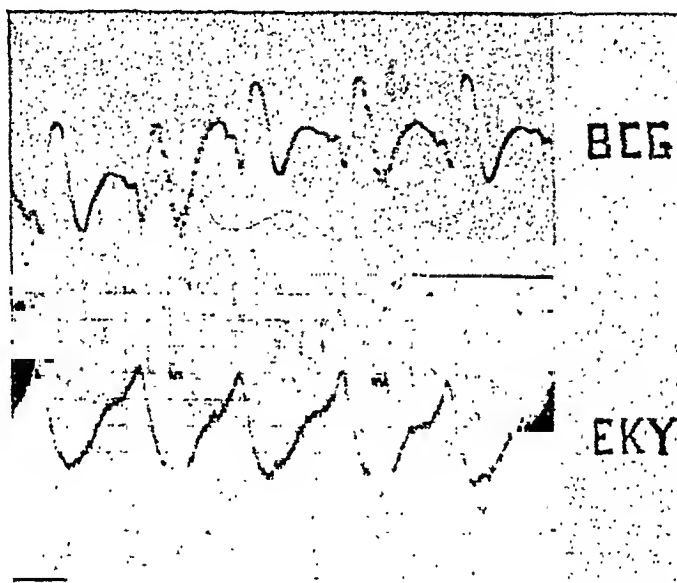


FIG. 2

The principles involved in the method used are best described by an example. When water is placed between the x-ray tube and the electrokymograph head, the amount of x-ray transmittance is indicated by the response of the string galvanometer resulting from moving the calibrator into the x-ray beam. The greater the depth of water the less the transmittance of x-rays and the smaller the calibrating response. As would be expected, a graph of these responses on semi-logarithmic paper gives a straight line (fig. 3). Thus, by means of the responses produced by the calibrator, one can measure any changes in the thickness of the water. Since muscle and blood absorb nearly the same amount of x-rays as water, it follows that one should be able to measure the dorso-ventral thickness of the heart by the method described above. First one calibrates with the electrokymographic (EKY) head over the ventricular

shadow. The head is then moved to the right of the spine shadow over chest walls and lungs only and the calibrations are repeated. In most cases, the transmittance of x-rays is so large when the heart is not in the field that the phantom for the heart (see above) must be placed between the x-ray tube and the subject. This absorbs x-rays to about the same extent as the heart. To determine the thickness of the heart, the points along the line on figure 3 which correspond to the calibrating responses over the heart on the left and over the chest and phantom on the right are found and the thickness of water to which these correspond is noted. Then, since the pressdwood of the phantom absorbs x-rays to the same extent as water or blood, its thickness (5 cm.) is added to that determined from figure 3. This gives the systolic thickness of the heart.

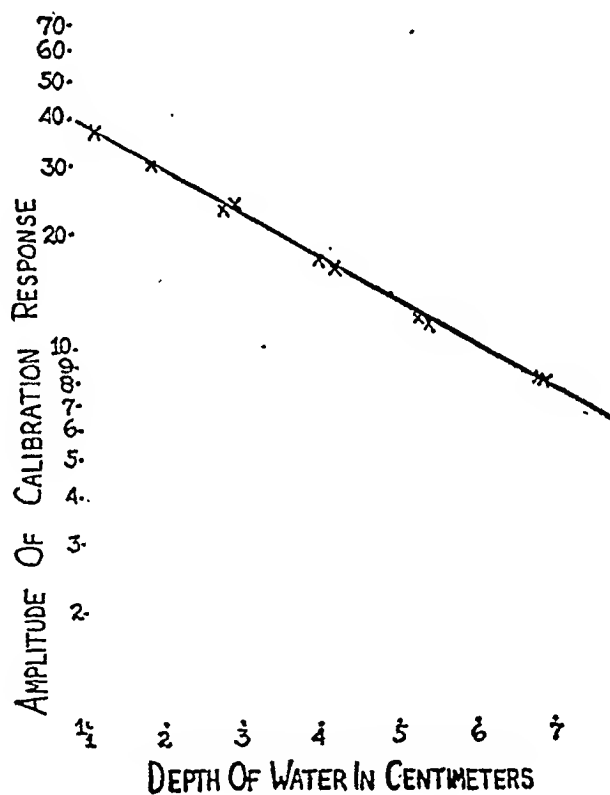


FIG. 3

When the EKY head is over the ventricles, it records the antero-posterior movements of the ventricular walls. If the calibrations are timed so as to occur at the end of systole, then they cause the string to move toward the position it takes during diastole. When the antero-posterior movements are small, a calibration will move the string beyond its diastolic position and with large ventricular movements, the calibration will not quite return the string to the diastolic level. In figure 2, the calibrations are shown for every other beat. The amplitude of the cardiac record is 1.06 times that of a calibration. Since a calibration corresponds to the x-ray absorption of 0.625 cm. of water, the change in AP thickness of the heart amounts to 0.66 cm. For small differences a linear relation between the calibrating response and the change in AP thickness may be assumed. For larger differences, it should be remembered that this is a logarithmic function.

The procedure followed in these experiments is as follows: The subject lies on the table with his feet firmly against the foot board. He is then told to take a deep breath and with fluoroscopic guidance, the EKY head is placed over the heart shadow and slightly nearer the apex than the base. Care is taken not to have any

TABLE 1

	DIASTOLIC THICKNESS	SYSTOLIC THICKNESS	STROKE CALCULATED FROM		% DEVIATION ¹
			EKY	BCG	
	cm.	cm.			
WOMEN					
SHE	5.98	5.35	88	84	+ 5
SPI	5.95	5.31	88	64	+27
WUR	6.27	5.52	107	105	+ 1
RYA	7.88	7.09	145	139	+ 4
TRU	5.48	4.93	70	85	-17
	5.42	4.90	66	82	-19
WEB	6.26	5.69	83	81	+ 2
VIT	5.67	5.00	87	102	-15
MIL	5.59	5.05	70	74	- 5
CAN	6.11	5.82	43	91	-53
	5.73	5.33	54	94	-42
PEA	6.27	5.63	93	89	+ 4
MEN					
FLA	7.78	7.22	102	110	- 8
MUS	6.57	6.04	82	116	-29
KEL	7.94	7.43	95	95	0
FRI	8.07	7.49	110	109	+ 1
McA	7.08	6.56	87	85	+ 2
DON	7.77	7.00	139	132	+ 5
DEL	7.49	6.86	110	112	- 2
DEA	6.52	5.89	95	86	+ 9
CUT	6.56	5.74	123	132	- 7
RAB	6.91	6.21	111	106	+ 5
POW	6.71	6.06	101	122	-17
	6.41	5.64	113	122	- 7
POL	8.52	7.92	121	116	+ 4
MYE	6.54	6.05	75	84	-11
LON	8.00	7.47	100	103	- 3
RIC	6.01	5.17	115	111	+ 4
	6.22	5.35	123	118	+ 5
CHA	6.43	5.66	113	100	+13
EVA, B.R.	7.73	7.15	106	86	+22
FIE	7.54	6.99	93	74	+32

¹ Difference between EKY and BCG results.

part of the head over the spine shadow. The amplification in the galvanometer circuit is adjusted to give a suitable record. After this the subject is allowed to breathe until the rate returns to normal. The subject is then instructed to take a deep breath and hold it while the ballistocardiograms and EKY densograms are simultaneously

recorded. The experimenter constantly watches the shadow of the galvanometer string and puts in a calibration at the end of every other systole. He records 2 strips of about 30 beats each during breath holding. Then the head is moved about 5 mm. away from what was judged to be the ideal position for recording. The purpose of this is to see whether the densograms recorded in the second position differ from those obtained first. A series of responses to calibrations over the chest walls and lungs to the right of spine is next recorded as indicated above. From this result and the calibrations over the heart the antero-posterior thickness of the heart is determined.

The x-ray tube was operated at 80 kv. and $2\frac{1}{2}$ ma. This produced 8 r per minute at the patient's skin. The exposure in the area under the heart averaged 3 minutes and on the right side of the chest $1\frac{1}{2}$ minutes. Since the x-rays were coned down as soon as the head was properly placed, the total exposure of any skin area was very

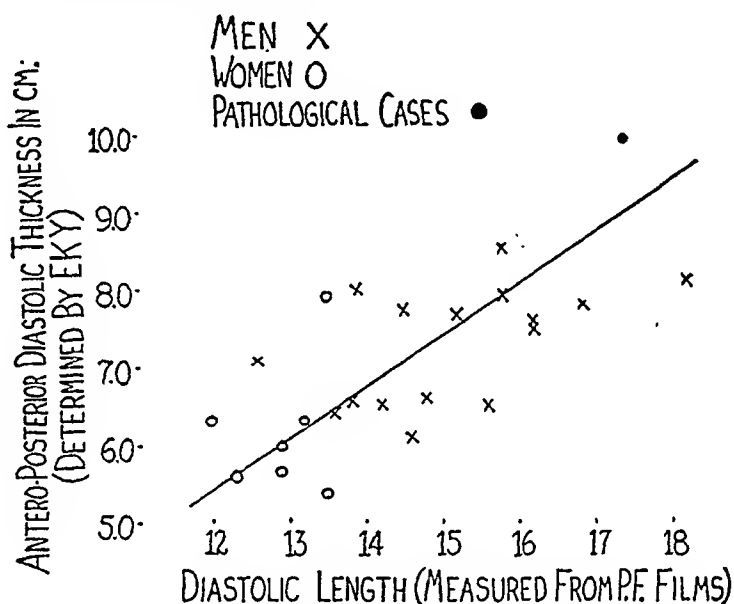


FIG. 4

small; therefore, frequent repetition of the procedure is possible without appreciable harm to the patient.

RESULTS

In table 1 is shown the thickness of the heart of each subject (medical students and nurses) in systole and diastole. These figures are a measure of the average thickness of that portion of the heart under the electrokymograph head. The measurements made from lateral roentgenograms would be larger than these, for then one is measuring from the right side of the heart which gives the ventral contour to the left side which makes up the dorsal contour. The thickness measured at any point on the anterior surface to a point directly posterior would be less than the figure obtained from such roentgenograms (10).

That our results are consistent with those determined from measurements of x-ray shadows is shown by figure 4. For this graph the heart shadow on a photofluorographic (PF) film has been enlarged to full size and the long diameter measured. One would expect considerable scatter in the results since these films were taken

sometime before the electrokymograph records and are not entirely satisfactory for measuring heart size. The results, nevertheless, do show a consistent trend.

To calculate the stroke volume (S.V.) from observations using the EKY, we have developed a formula which would give the best fit with the ballistocardiographic results. This formula is $S.V. = 12.2 (T^2 \text{ diast.} - T^2 \text{ syst.})$ where T is the antero-posterior thickness as measured with the electrokymograph. If the heart were a

TABLE 2. THE ANTERO-POSTERIOR DENSITY CHANGE PER CALIBRATION

	POSITION 1	POSITION 2	AV.	% DEVIATION
WOMEN				
CAN	.50	.51	.505	1
SHE	.98	1.03	1.005	2
WUR	1.12	1.27	1.195	7
SPI	1.04	1.03	1.035	1
TAY	.70	.60	.65	8
ROU	.86	.87	.865	1
RAM	.93	1.12	1.025	8
VIT	1.07	1.08	1.075	1
WEB	.99	.92	.955	4
MEN				
CAR	1.28	1.40	1.34	4
PIN	1.29	1.35	1.32	2
COO	1.06	1.02	1.04	2
TRU	.87	.84	.855	2
RUS	.84	.76	.80	5
SOU	.82	.82	.82	0
SHE	.45	.48	.465	3
STE	1.51	1.66	1.585	5
PAU	.59	.75	.67	12
MIL	.87	.86	.865	1
MUS	.94	.77	.855	10
MYE	.82	.73	.775	6
LON	.90	.78	.84	7
POW	1.04	1.24	1.14	9
RIC	1.35	1.40	1.375	2
FRI	.91	.96	.935	3
CHA	1.26	1.18	1.22	3
EVA	.97	.86	.915	6
FIE	.88	.88	.88	0
FLA	.92	.87	.895	3
DEA	.88	.93	.905	3
CUT	1.17	1.22	1.195	2

sphere in systole and diastole and if one were measuring the true diameter, then the exponent for T should be 3. Neither of the above premises is correct, and it turns out that our best fit is obtained using the exponent 2.

One questions whether the electrokymographic records are reproducible. To test this, we have moved the head away from the position considered most satisfactory. In table 2, the results are shown. The differences are not in every case due

entirely to change in position of the head. In a number of subjects, the output of the heart changed and accounted for part of the difference. Furthermore, in some subjects the calibrations were not placed so that they always included the end of systole. Thus, there were occasionally too few calibrations to give a reliable average. An automatic device to calibrate at the proper time would, we feel, reduce the differences shown in this series, which now average 4 per cent.

The position of the heart within the chest changes during each systole and one must consider whether this has an important effect on the records. When recording the movements of the lateral borders of the heart, positional changes play a large part in the results obtained. With the EKY head placed at about the middle of the ventricular shadow, the positional movements have far less effect though the rotation of the heart to the right probably means that more of both the right and left ventricles come under the EKY head. Furthermore, the base of the heart moves toward the apex during contraction. Thus, the recorded movement may well be less than the antero-posterior change in thickness of the heart at any point on its surface.

Another source of the difficulty is the chest calibrations. It is impossible to be sure that the EKY head covers the same amount of the shadow of ribs, muscle and lungs on the right and on the left. Nevertheless, this does not seem to result in any large error. In each subject we have made chest calibrations in 2 positions. One is a centimeter cephalad of the other. In our calculations of output we have averaged these calibrations. If instead one uses one of the extremes the maximal difference in output is found to be 3 per cent. In most cases it averaged less than 1 per cent.

The differences between our results and those obtained using the ballistocardiograph are small (table 1). Eighty per cent of the EKY results differ from ballistic calculations by less than 20 per cent. The ballistic method checks with the Fick (8) no better than this. Therefore, it is possible that the EKY gives results as good as or better than the ballistocardiograph. The real test of the method will come when it is checked against the Fick or the Stewart method.

DISCUSSION

The strokes given in table 1 were not obtained under basal conditions. The subjects were not post absorptive, had not rested for half an hour before the observations were begun, and furthermore the stroke of the heart was affected by suspension of respiration. In this preliminary work, we were interested only in comparing the output by the two methods. If our method proves satisfactory, it can be adapted to basal conditions and in many individuals will not require breath holding.

One may question whether the EKY head is over the ventricles only and is therefore recording their movements alone. If the subject were standing, the left atrium would extend so far downward that a part of it would probably lie between the x-ray tube and the EKY head. With subject supine, the apex drops far enough so that the atrium probably does not intercept the x-rays activating the EKY. Even if part of the x-ray beam is intercepted by the left atrium, the movement of the base of the heart toward the apex during ejection elongates the atria and may balance out any effect on antero-posterior thickness of these chambers which is produced by the entrance of blood (12).

We are now recording densograms with the subject in the left lateral position as well as supine. In the lateral position the apex probably moves downward enough so that here also the atria do not interfere with the ventricular recording. With measurements of movements and thickness in two dimensions, the accuracy of the methods is increased. Furthermore it is probable that we shall be able to calculate heart size from these records. If so, another means of determining the importance of Starling's Law in the human heart will be available; for evidence in dogs see (11).

A well nourished ventricle, working against a normal arterial pressure and receiving an adequate venous inflow, contracts considerably during ejection of blood. On the other hand, a ventricle which is in poor condition is able to contract very little and must dilate to maintain an adequate output. In a group of healthy individuals, if athletes with thick walled ventricles were omitted, we might also find that those whose hearts were large for the size of their bodies, had ventricles which contracted

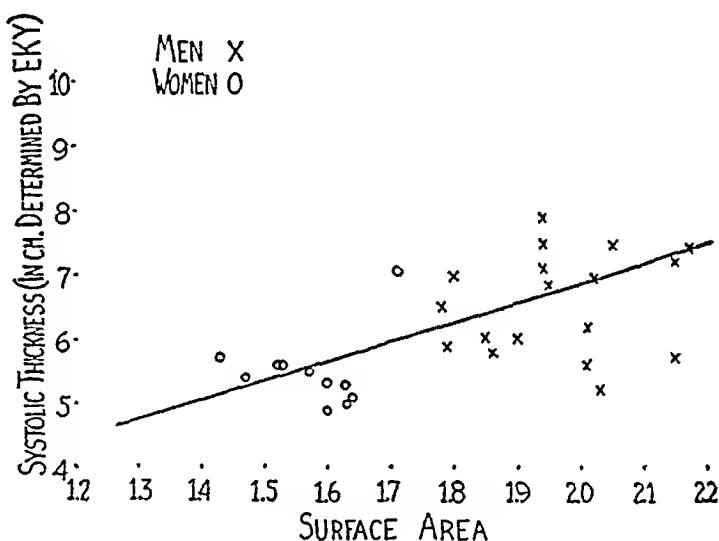


FIG. 5

little during ejection of blood. To test this, the systolic thickness of the heart of each of our subjects was plotted against his surface area (fig. 5). A line was then drawn on this graph which showed the average thickness of the heart for persons of any size. From this graph and the AP thickness of each person's heart, the per cent of predicted heart size was calculated. Those whose hearts were 80 to 90 per cent of the predicted size showed an average change in thickness during systole of 11.2 per cent; hearts 90 to 100 per cent of normal size showed a change of 9.3 per cent; those 100 to 110 per cent of normal showed an 8.6 per cent change and those 110 to 120 per cent of normal showed an 8.0 change. Thus, the size of a heart and its ability to contract are related. The results suggest that change in thickness of the heart with ejection may be a measure of cardiac condition.

CONCLUSIONS

A method for measuring the output of the heart using the EKY has been described.

This method gives a measure of the AP thickness of the heart both in systole and diastole (see table 1).

An inverse relation between thickness of the heart and its contractile power is suggested from these observations.

We wish to express our thanks to Doctor George C. Henny and Doctor Robert R. Newell of the Department of Radiology, Stanford University Hospital, San Francisco, Calif., who offered suggestions during the carrying out of these experiments.

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AN EVALUATION OF THE CARDIOVASCULAR EFFECTS OF CERTAIN DRUGS IN HYPOTENSIVE DOGS¹

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NUMEROUS attempts have been made to find drugs that would be beneficial in the treatment of hypotensive states and 'shock' (1-4). The majority of studies have been confined to the effects of various pressor agents on arterial and venous pressures or on survival time. As Frank *et al.* (1) have stated, "it has been assumed that with an increased arterial pressure resulting from vasoconstriction there is a redistribution of blood through the critical circuits." Measuring the effects on arterial pressure caused by a particular drug gives no information on whether or not blood is being distributed to critical circuits, since variations in arterial pressure are reflections of the summation of changes in the peripheral vascular bed (vasoconstriction or vasodilatation) and cardiac output. For example, if a given drug produces an increase in arterial pressure and an increase in cardiac output, with a decreased total peripheral resistance (peripheral vasodilatation), the efficiency of such a drug in effecting satisfactory circulation to vital organs of the body can be assumed to be higher than one which produces an increase in blood pressure due to an increase in total peripheral resistance (peripheral vasoconstriction) alone.

With these concepts in mind the effects of Aranthal (2-methylamino-6-hydroxy-6-methyl heptane 2-methylamino iso-octanol), Oenethyl (2-methylamino heptane), epinephrine, amphetamine and theophylline on cardiac output, total peripheral resistance (T.P.R.), as well as mean blood pressure were examined in hypotensive dogs.

METHODS

Dogs anesthetized with 300 mg/kg. of sodium barbital were bled rapidly from a femoral artery until the mean blood pressure was approximately 50 mm. Hg, as determined by a mercury manometer in the other femoral artery. They were maintained at this level for about 90 minutes with additional bleeding or infusion of blood as needed. Usually the blood pressure stabilized within 15 to 30 minutes after the initial bleeding. The drug to be tested was given intravenously at the end of the 90-minute period and cardiac outputs determined for a varying length of time, usually 120 minutes. At the end of the experiment the dogs were transfused to determine whether or not irreversible shock had been precipitated. Dogs found to be in the irreversible stage are not included in this report.

Cardiac outputs were determined from pressure pulses obtained from the right carotid artery by the method of Hamilton and Remington (5). This method has

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been found to give satisfactory results when compared to the direct Fick method (6). Total peripheral resistance was calculated by the formula (7)

$$\text{TPR} = \frac{\text{Pm} \times 1332}{\text{C.O./sec.}} = \frac{\text{dynes sec.}}{\text{cm}^5}$$

The figures given for cardiac output are expressed in percentages of values at the end of the hypotensive period or values immediately before a drug was administered.

RESULTS

The effect of Aranthal was observed in 12 hypotensive dogs. A single dose of 10 mg/kg. consistently produced a prompt and prolonged increase in cardiac output (fig. 1). The average increase in cardiac output was about 20 per cent and the duration of action from 85 to 240 minutes. Changes in T.P.R., mean pressure, and heart rate are illustrated in figure 1. In the hypotensive dog 20 mg/kg. appears to produce a maximal increase in cardiac output. Further doses then fail to produce any detectable change until the output begins to fall. When this occurs Aranthal will again augment the output.

TABLE 1. EFFECTS ON CARDIAC OUTPUT

DRUG	AVERAGE % CHANGE FROM END OF HEMORRHAGE	AVERAGE DURATION OF ACTION, MIN.
Aranthal.....	+21	120
Oenethyl.....	+15	60
Epinephrine.....	+20	3
Amphetamine.....	+10	120
Theophylline.....	-35	60

Oenethyl (10 dogs) in a dosage range of 2.5 to 10 mg/kg. increases the cardiac output, but the effect is more irregular and less prolonged than with Aranthal. The mean pressure change is variable and the T.P.R. decreases. Successive doses of Oenethyl evoke decreasing responses from the cardiovascular system.

The administration of amphetamine (7 dogs) in doses of 1 to 5 mg/kg. produced an average increase in cardiac output of 10 per cent for the 120-minute period. A small but consistent fall in mean pressure was observed, and the T.P.R. fell below the hypotensive level after an initial steep rise (fig. 2).

Theophylline (8 dogs), 5 mg/kg., characteristically produced a considerable decrease in cardiac output, a drop in mean pressure and a small increase in T.P.R.

A prompt and marked rise in cardiac output, T.P.R., mean pressure and heart rate occurs when epinephrine, 0.05 mg/kg., is administered. The duration of these effects is brief, usually less than 5 minutes.

DISCUSSION

The cardiac output in hypotensive dogs was increased by Aranthal, Oenethyl, epinephrine, and amphetamine. The comparative effects of these drugs on cardiac output are presented in table 1. As one would anticipate, the duration of action of

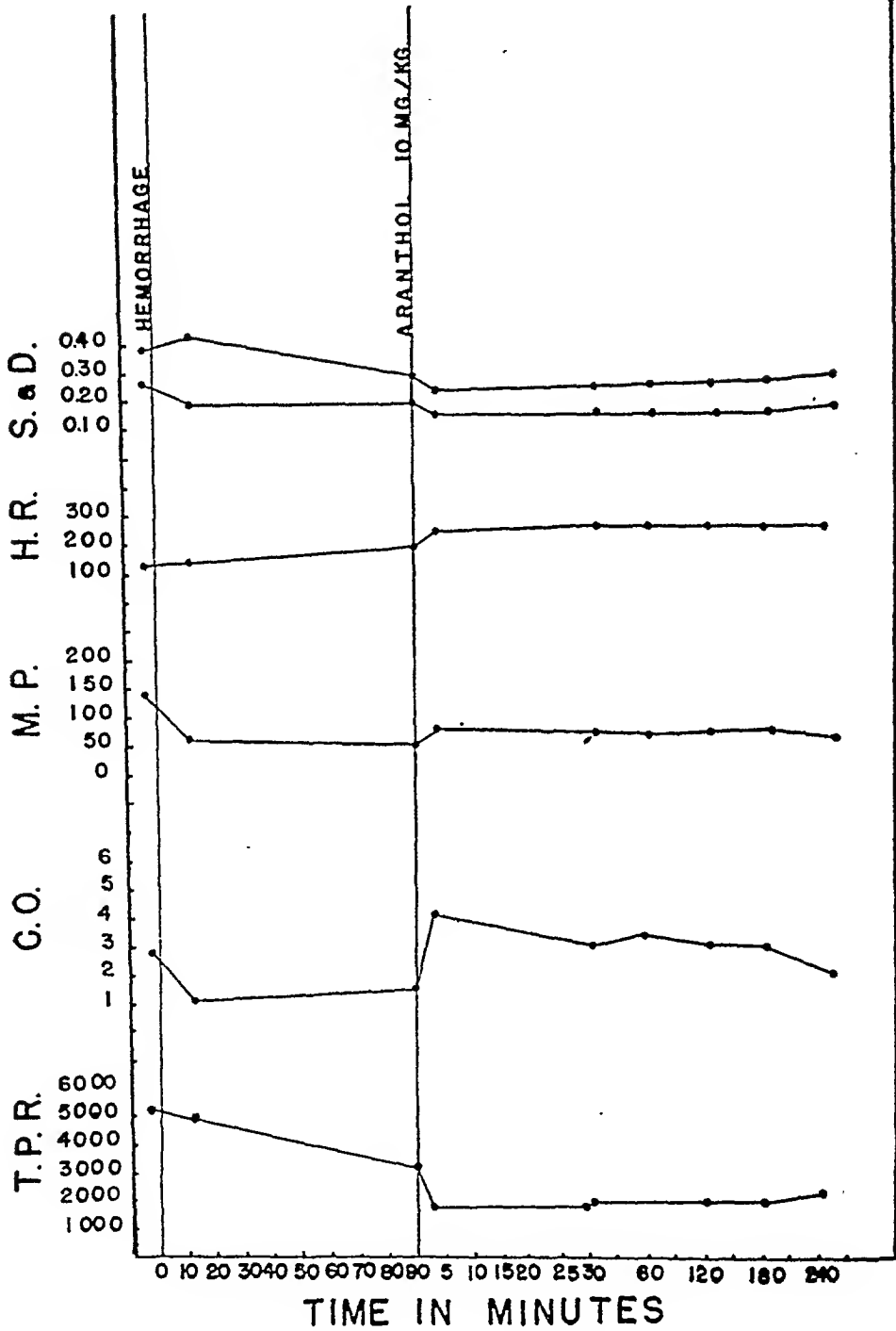


Fig. 1. THE FIRST SET OF POINTS gives the control values. Hemorrhage occurred at 0 time. T.P.R., total peripheral resistance in absolute units; C.O., cardiac output in liters/min./M² body surface; M.P., mean pressure in mm. Hg; H.R., heart rate; S. & D., systolic and diastolic duration.

epinephrine is transitory. Theophylline produced a decrease in cardiac output of such magnitude as to result in a fall in mean pressure despite an increase (slight) in T.P.R.

Amphetamine, after a latent period of about 5 minutes, increased cardiac output appreciably. However, there is always a preliminary decrease below the hypotensive

level, and further, the mean pressure for 15 to 30 minutes after the injection of amphetamine falls to a very dangerous level. It is of interest to note that the effect

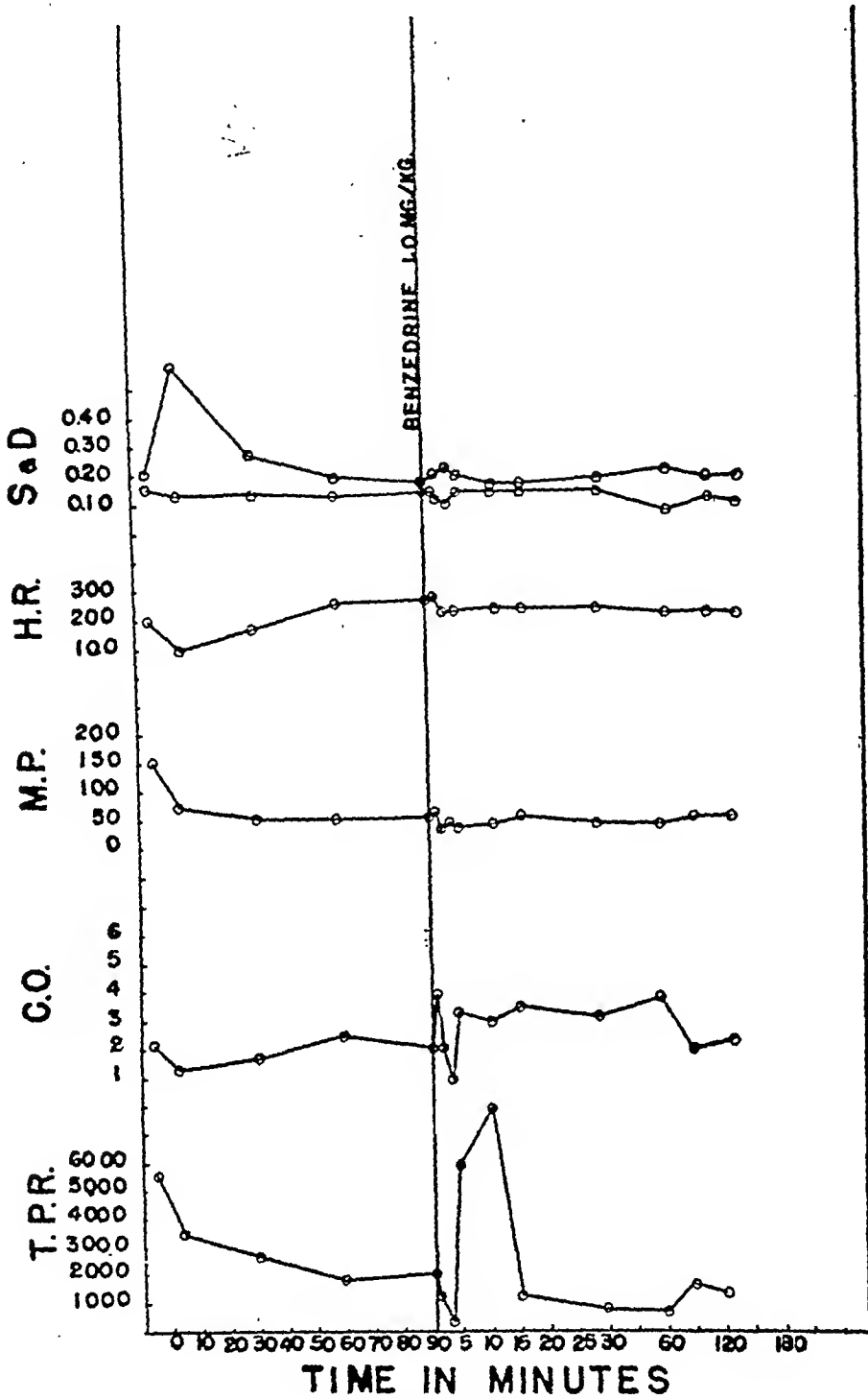


Fig. 2. SYMBOLS the same as in fig. 1

of amphetamine in the hypotensive dog is the direct opposite of that expected from results on normal dogs.

Oenethyl produces a significant and prolonged increase in cardiac output, but the increase is more erratic than that produced by Aranhol. Also, successive doses have

a decreasing effect on output. As Jackson (8) and Ahlquist (9) have shown tachyphylaxis occurs, limiting the usefulness to the first dose.

Aranthol has been reported (10-12) to cause a marked and prolonged increase in force of contraction and heart rate with an increase in blood pressure. In the hypotensive dog there is a marked increase in cardiac output which is associated with a decrease or only slight increase in T.P.R. Therefore, the improved circulatory dynamics appear to be largely cardiac in origin, and with no peripheral vasoconstriction; the improved circulatory efficiency should be reflected throughout the vascular system. The onset of the effects of Aranthol are almost immediate and the duration of its action is prolonged. The properties suggest that Aranthol might be of value in the treatment of hypotensive conditions.

The data on the duration of systole and diastole yielded little pertinent information. In about 60 per cent of the dogs, during hemorrhage, a marked increase in the length of diastole and decrease in systolic (figs. 1 and 2) duration occurred.

SUMMARY

Aranthol, Oenethyl, epinephrine, amphetamine and theophylline were administered intravenously in varying doses to dogs rendered hypotensive by bleeding to 50 mm. Hg for 90 minutes. Data on the effects of these drugs on total peripheral resistance, cardiac output, mean pressure, heart rate and the duration of systole and diastole are presented. Aranthol, Oenethyl, amphetamine and epinephrine increase the cardiac output in hypotensive dogs, while theophylline consistently reduces the output. The effects of Aranthol and Oenethyl are greater and more sustained than any of the other drugs tested. Oenethyl produces a more erratic response and, in addition, tachyphylaxis occurs which is not apparent with Aranthol.

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EXCRETION OF SOLUTES AND OSMOTIC WORK OF THE 'RESTING' KIDNEY OF HYDROPENIC MAN

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THE energy requirement for the production of urine was a subject of great interest to the early workers in renal physiology. In the absence of exact knowledge of the path of urine production, and of the intermediate processes of renal metabolism, they were led to consider first the thermodynamically necessary work required to produce the differences in solute concentration between plasma and urine (1, 2). On the basis of general principles of thermodynamics and the gas laws the formula was derived

$$W = RT V (U \ln U/P + P - U) \quad (1)$$

where W indicates the work, R is the gas constant, T the absolute temperature, U the urinary and P the plasma concentration of a solute and V is the urinary flow. (See also Newburgh, 3.) The work value thus derived refers to the idealized process of urine formation conducted in a thermodynamically reversible manner, without energy losses due to back diffusion, heat production, metabolic side processes or irreversible reactions, in short at 100 per cent efficiency. It represents the theoretical minimal work necessary to produce urine. It was pointed out by Rhorer (2), that this work consisted not alone in producing from plasma a fluid of differing total osmolality, but that the concentrations of each individual solute had to be considered. Later, Borsook and Winegarden (4) calculated in a more exact manner than preceding workers the theoretical osmotic work of urine production. Most recently Newburgh has presented a discussion of changes which tend to reduce the renal osmotic work. Obviously there are two theoretical minima for renal work: 1) if urine and plasma are of the same composition, in which case no osmotic work (but still filtration work) need be done; and 2) if urine excretion ceases. Newburgh (3) pointed out that changes in disease, such as increases of blood urea, tended to decrease the osmotic work required to clear the body of a given quantity of solute.

As part of a general inquiry into the osmotic limitations of the kidney it appeared of interest to investigate experimentally the renal work during hydropenia and osmotic diuresis. The following questions, among others, were posed: Is there an over-all maximum of renal osmotic work during hydropenia and forced diuresis? If so, is it the same for loading with different solutes? Under what conditions of urine flow does such a maximum occur? Is there any relation between urine osmolality and renal osmotic work performed or the work capacity? The present communica-

tion deals with the pattern of solute excretion and the renal osmotic work of hydropenic man, under 'resting' conditions, i.e., without solute loading.

METHODS

The subjects were boys 8 to 15 years of age with normal renal function and without major disease. They were fasting and had received no water for 16 hours prior to experimentation. One or two blood samples were obtained on each subject during the one or two periods of urine collection which extended for approximately 30 minutes. Plasma and urine were analyzed for urea, sodium, potassium, chloride, phosphate, and in some instances sulfate. On urine the total osmolality was also determined. The methods and calculations used have been described (5).

RESULTS AND DISCUSSION

Solute Excretion. In table 1 are given the mean values with their standard errors of the concentration of solutes in plasma and urine of 15 subjects, who were studied over 26 periods of urine collection in 17 experiments. Also given is the mean value for urine flow, corrected to 1.73 m² body surface. It may be readily seen that urea accounted for about one-half and the electrolytes for the other half of the osmolality of urine. The sum of the solutes determined accounted on the average for 84 per cent of the total osmolality, calculated from the freezing point depression by a procedure outlined previously (5). The magnitude of individual variations of each of the urine solutes should be pointed out. The urine flow, as well as the concentration of electrolytes, ranged from values of 0.5 to 1.5 of the mean. Urea was the most constant solute, with about two thirds of the variation of the electrolytes. Some of the variation is undoubtedly due to differences in the preceding dietary intake.

The plasma values other than the total calculated osmolality deserve little comment. Its value was calculated by the procedure outlined in the footnote to the table. A similar value is obtained if the normal value for CO₂ content, 27 m.Osm/l, and for the sum of Ca and Mg, 3.5, is added to the sum of the solutes determined.

A comparison of the data for urine with those in the literature is of interest. Similar rates of flow have been observed by McCance (6) and Gamble (7), but Chesley (8) under conditions of prolonged thirsting and fasting observed smaller volumes. The total osmolality, as well as the concentration of the individual solutes, is similar to the values measured by McCance (6) on two adult subjects, but lower than the maximum achieved during a 6-day period of water deprivation in the experiments of Gamble (7).

Osmotic Work. In table 2 are summarized the data on the minimal renal work of the subjects, calculated by *equation 1*. An inspection of the distribution of the values indicates that urea accounted for the bulk, approximately two thirds, of the work. Potassium was next in importance. Together these two solutes accounted for 90 per cent of the total work. Sodium and chloride were lowest in the list. It is of interest to contrast the distribution of the work values with that of the concentrations. By the nature of the equation it is the ratio between plasma and urine, rather than the absolute urine concentration, that determines the renal work.

To evaluate the significance of the total work value, reference should be made to the work of the kidney under other conditions. In osmotic diuresis during hydro-

TABLE 1. PLASMA AND URINE CONCENTRATIONS OF SOLUTES
Means and standard errors of 26 periods on 15 subjects in 17 experiments.
Concentrations are expressed in milliosmols per liter.

SPECI- MEN	URINE VOL. cc/min/ 1.73 M ²	UREA	Na	K	Cl	PO ₄	SO ₄	SUM SOLUTES DETER.	TRUE TOTAL OSMOLARITY CALC.
Urine	0.49±0.03	500±17	153±12	143±10	162±12	38.0±3.0	26.3±0.8	986 ±23	1182±18
Plasma		7.3±0.4	148±2.0	4.9±0.2	105±1.1	1.5±0.1	1.1±0.1	267.8±3	304 ¹

¹ The value for total osmolality of the plasma was estimated as 2(Na + K) - 8 + (urea). The formula is based on the simplifying assumption that the proteins are the only osmotically negligible ions and that urea is the only non-electrolyte. The total osmolality of the electrolytes expressed in terms of cation equivalents is 2(Na + K) + 1.5(Ca + Mg) - (Prot.). Substitution of the values of 7 mEq/l. for the sum of Ca and Mg, and of 18 for protein, leads to the previous expression.

TABLE 2. IDEAL OSMOTIC WORK OF RESTING HYDROPEMIC KIDNEY¹
Means and standard errors of 26 experimental periods. Work values are expressed in cal/min/173 M²

URINE FLOW cc/min/ 1.73 M ²	UREA	Na	K	Cl	PO ₄	SO ₄	TOTAL ² WORK	WORK/CC. URINE
0.49±0.03	0.47±0.03	0.004±0.001	0.10±0.01	0.006±0.002	0.024±0.002	0.018±0.003	0.63±0.03	1.30

¹ Work was calculated according to equation 1. Plasma values were each multiplied by 1.05 to give an assumed value for the concentration in the glomerular filtrate. For expression of the work in cal/min. the concentration of each solute in plasma and urine was expressed in osmols/l., and the urine volume, corrected to 1.73 M² body surface, in l/min. A value of 1.986 cal. was used for K, and 310° abs. for T.

² The value includes 0.008 cal/min., for the combined work for HCO₃ and glucose calculated on the assumption of a value for their sum of 25 m.Osm/l., in the plasma, and zero in the urine.

penia, even though the urine osmolarity decreases, the calculated work rises to 4 cal/min., about 7 times as high as the value in the present experiments. A second question is how the work in the hydropenic 'resting' state compares with that under normal, 'eu-hydrouric' conditions of urine flow. Such a comparison is presented in table 3. It contains a series of work values for different rates of urine flow, calculated under the simplifying assumption of a constant solute load and constant plasma concentration of the solutes. Such an assumption introduces an error only with respect to urea. The urea work with rising urine flows is under-estimated, since with hydration the plasma concentration of urea decreases and the clearance increases. It may be seen from the table that with flow increasing to the eu-hydrouric range, the total work value diminishes slightly to reach a minimum at 2 cc/minute. It rises again slightly with further increase in volume. The relative constancy of the total work is a consequence of compensating changes in the values for the different solutes. As the work for Na, Cl, and the sum of bicarbonate and glucose rises, that for the other solutes falls. The general pattern of work curves for constant load and variable volume has been described schematically by Newburgh (3).

TABLE 3. CALCULATED OSMOTIC WORK FOR CONSTANT SOLUTE LOAD AND PLASMA CONCENTRATIONS AND VARYING URINE FLOW¹
Values are expressed in cal/min/1.73 M²

URINE FLOW cc/min/ 1.73 M ²	UREA	Na	K	Cl	PO ₄	SO ₄	GLUCOSE ² AND HCO ₃	TOTAL
0.5	.47	.004	.10	.006	.024	.018	.008	.630
1.0	.39	.015	.075	.003	.018	.012	.015	.528
1.5	.33	.044	.059	.016	.014	.009	.023	.495
2.0	.28	.078	.048	.036	.011	.007	.031	.491
2.5	.25	.116	.037	.059	.009	.006	.038	.515

¹ The solute load was assumed to consist of the average values determined for the condition of hydropenia. ² See footnote 2 in table 2.

One may conclude that under the conditions here described, i.e. of maximal osmolarity and minimal urine volume, the kidneys are in a relatively resting state. Their thermodynamic work exceeds only little that done under eu-hydrouric conditions.

The absence of relationship between urinary osmolarity and renal work should be emphasized. In the literature it has been frequently assumed, either expressly (6) or by implication (9), that a maximal urinary concentration is either synonymous with, or indicative of, a state of maximal renal work. Such an assumption is not only contradicted by the results of the present study but is actually theoretically unjustified. The factors defining the maximal urinary concentration or the ratio between urine and plasma osmolarities are as yet unknown. They may be a function of the thermodynamic work necessary to produce a given concentration difference, or they may be related to an over-all osmotic ceiling, possibly dependent on the permeability of the distal tubular wall. Even if the first case held, the concentration difference by itself could not be a measure of the work being done. The situation would be closely comparable to the case of a concentration cell. The con-

centration difference, i.e. the potential, by itself no measure of the energy output, has to be multiplied by flow (current) to yield a work value. Although under conditions of minimal urine flow, as under conditions of minimal electrical flow, the potential is maximal, the output is only a fraction of the possible maximum. If the second hypothesis is applied, namely that an over-all osmotic ceiling related to the structure of the distal tubular wall governs the maximum osmolarity, no conclusion at all can be drawn from the osmolarity with regard to the total osmotic work.

The question as to whether or not one can predict the maximal work value, i.e. the work capacity, from that of the resting kidney cannot be answered as yet. Off-hand there is no relationship apparent but it remains for further studies to determine whether such an association exists, and what its nature may be.

Finally the relation of the theoretical work to the actual metabolism of the kidney should be discussed. It was stressed in the introduction that the calculated work refers to a thermodynamically reversible process, conducted at 100 per cent efficiency. Actually, the thermodynamic work has been found in various experimental animals to account for only 1 to 2 per cent of the total metabolism of the kidney, as measured by its oxygen consumption (10-12). No direct measurements of renal efficiency are available in man. An approximate estimate may be given on the basis of the reported oxygen consumption of the 'resting' human kidney, determined to be 10 cc/minute by Bradley and Halperin (13). Assuming a R.Q. of 1.0, so that 1 cc. of oxygen corresponds to 5 cal., the total energy available to the human kidney would then amount to 50 cal. The average value of thermodynamic work here reported, 0.6 cal/min., corresponds to 1.2 per cent of the total energy, a value in agreement with the previously cited figures for experimental animals. A similar estimate of the efficiency of the human kidney was reached in an indirect manner by Borsook and Winegarden (4). Suffice it to say here, that there exists considerable doubt as to whether any relation obtains between the thermodynamic work and the total metabolism of the kidney.

SUMMARY

The solute excretion and the renal osmotic work in man in the hydropenic state has been presented. It is concluded that the kidney under conditions of minimal urine flow and maximal osmolarity is in a relatively resting state. The osmotic work is only little more than that under normal, eu-hydroureic conditions.

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EXCRETION OF SOLUTES AND OSMOTIC WORK DURING OSMOTIC DIURESIS OF HYDROPENIC MAN. THE IDEAL AND THE PROXIMAL AND DISTAL TUBULAR WORK; THE BIOLOGICAL MAXIMUM OF WORK

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PREVIOUS papers dealt with the relations between urine flow and excretion of solutes in hydropenic man during osmotic diuresis produced by 11 different loading solutes (1, 2). In this communication different aspects of these experiments will be presented. The pattern of excretion of the individual loading solutes, the relations between urine and plasma concentrations, and the electrolyte losses will be considered. Furthermore, calculation of the renal work, both of the ideal process and of the work for the proximal and distal portions of the renal tubule, is included.

METHODS

The experimental procedures and the chemical methods used have been described previously (1, 3). The material of this paper includes 214 urine collection periods observed during 26 experiments on 21 subjects, of whom 3 were diabetic. Five of the subjects were studied twice. All loading solutes except xylose, sorbose and sorbitol were used in more than one experiment each. Glucose loading was carried out only in diabetic subjects. Mannitol was used as the loading solute in 5 experiments and creatinine in 3. In several of the experiments with NaCl, creatinine and glucose, a large dose of solute was given orally prior to its intravenous administration. In one experiment urea was given orally followed two hours later by glucose both orally and intravenously. In another experiment the order of administration of the two solutes was reversed. For calculation of the dose of the electrolytes in osmoles, the molecular weight was divided by the number of ions constituting the molecule.

The calculation of the ideal renal osmotic work by the equation

$$W = RT V (U \ln U/P + P - U) \quad (1)$$

where W indicates the work, R is the gas constant, T the absolute temperature, U the urinary and P the plasma concentration of a solute and V is the urinary flow has been described previously (3, footnote 1, table 2).

RESULTS

Pattern of Solute Change in Plasma and Urine during Osmotic Diuresis

In table 1 are summarized representative experiments on 8 of the 11 solutes studied. They are: urea, creatinine, NaCl, sodium sulfate, sodium para-aminohippurate, mannitol, sucrose and glucose. Omitted are loading experiments with

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TABLE 1. URINE FLOW AND SOLUTE CONCENTRATIONS IN PLASMA AND URINE DURING OSMOTIC DIURESIS PRODUCED BY VARIOUS LOADING SOLUTES

All concentrations are expressed as m. Osm/l.

C. B., 11 yrs., 28.9 kg., 1.08 M²

UREA

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/MIN/ 1.73 M ²	UREA	Na	K	Cl	PO ₄	SO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
P-2 U	-47 to -5	0.50	552.1	225.0	106.5	172.2	34.8	23.3	1114	1179
P	-14		8.4	145.5	5.8	103.6			263	303

0 to 15 I. V. injection 52% urea, 1385 m.Osm/1.73 M² in 46 cc. saline

2 U	38 to 59	4.58	556.7	50.0	15.8	37.0	4.1	2.3	666	686
P ¹	29		53.7	149.2	8.8	106.4		1.2	319	362
P ²	48		50.1	146.2	6.5	102.8		1.1	307	348
3 U	59 to 78	4.61	561.0	42.5	15.0	33.0	5.4	2.6	660	700
P	68		47.0	144.7	5.0	106.4		0.9	304	338

G. P., 13 yrs., 42.5 kg., 1.34 M²

CREATININE

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/MIN/ 1.73 M ²	CREATININE	UREA	Na	K	Cl	PO ₄	SO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
P-1 U	-101 to -66	0.66		466.0	67.5	125.3	112.5	41.6	26.9	840	1029
P	-87			6.8 ¹	150.0	4.9	103.6	1.6		267	309

0 to 4 I.V. injection 7.6% creatinine, 144 m.Osm/1.73 M² with 50 cc. saline

3 U	5 to 25	6.97	261.2	143.8	52.5	39.5	79.0	8.4	4.6	589	661
P	15		8.8	6.8 ¹	146.3	5.0	102.8			270	310
4 U	25 to 46	3.51	276.2	183.9	65.0	46.3	87.0	11.3	6.6	676	756
P	35		6.5	6.8 ¹	150.0	5.7	102.0			271	317

48 to 56 I.V. injection 7.6% creatinine 218 m.Osm/1.73 M²

5 U	58 to 79	7.96	273.2	79.0	50.0	34.0	73.0	5.5	3.0	518	632
P	68		15.3	6.8 ¹	148.5	5.4	103.2	1.7		281	322
6 U	79 to 100	4.97	315.8	120.0	45.0	39.0	72.6	8.4	4.7	606	710
P	89		11.1	6.8 ¹	145.5	5.3	102.8			272	312

IV. H., 11 yrs., 31.2 kg., 1.12 M²

NaCl

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M ²	Na	Cl	UREA	K	PO ₄	SO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
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0 to 27.5 I.V. injection 10.4% NaCl, 1052 m.Osm/1.73 M² with 30 cc. saline

1 U	71 to 100	8.36	275.0	262.0	78.9	24.5	6.1	3.5	650	684
P	85		168.8	130.8	4.8	3.5	1.3	1.2	310	341
2 U	100 to 121	6.34	255.0	249.6	67.0	35.2	5.2	3.1	615	666
P	112		170.0	129.6		4.4		1.4	305	346
3 U	121 to 142	6.34	247.5	241.0	68.9	43.8	4.8	2.8	608	728
P	141.5		166.2	124.0	4.7	3.7	1.3	1.1	301	336

TABLE 1—Continued

L. R., 14 yrs., 38.0 kg., 1.30 M²Na₂SO₄

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M ²	UREA	Na	K	Cl	PO ₄	SO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
<i>0 to 6 I.V. injection 18% Na₂SO₄, 758 m.Osm/1.73 M² with 50 cc. saline</i>										
1 U	19 to 47	8.94	61.1	355.0	41.5	8.0	2.5	196.2	664	708
P	30		7.4	167.0	6.2	100.0	1.2	12.6	294	346
2 U	47 to 66	5.31	88.8	393.0	63.0	4.6	3.6	175.7	729	779
P	57		8.1	167.0	6.2	102.4		8.6	292	346
<i>71 to 78 I.V. injection 18% Na₂SO₄, 506 m.Osm/1.73 M²</i>										
3 U	81 to 107	10.6	45.9	368.0	35.8	9.8	1.9	194.8	656	690
P	91		7.0	179.0	6.5	102.0		13.9	308	370
4 U	107 to 127	7.45	70.6	395.0	53.3	2.0	2.9	187.2	711	772
P	117		7.3	170.0	5.6		1.2	11.0	195	350

A. R., 10 yrs., 28.9 kg., 1.02 M²

Na p-aminohippurate

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M ²	PAH	UREA	Na	K	Cl	PO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
<i>0 to 2 I.V. injection of 20% NaPAH, 113 m.Osm/1.73 M²</i>										
2 U	21 to 41	2.77	240.0	110.5	248.8	31.4	29.4	23.2	683	819
P	29		2.8	7.3	151.5	5.7	104.0		271	314
<i>49 to 51 I.V. injection of 20% NaPAH, 220 m.Osm/1.73 M²</i>										
4 U	74 to 95	4.35	232.0	91.8	248.8	26.8	41.4	14.2	655	684
P	84		6.7	6.0	159.8	5.8	101.2	1.3	281	329
5 U	95 to 122	3.52	238.4	110.6	240.0	43.8	38.6	19.7	691	742
P	107		4.6	6.2	159.0	5.7	100.8		276	328

A. R., 10 yrs., 28.9 kg., 1.02 M²

MANNITOL

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M ²	MANNITOL	UREA	Na	K	Cl	PO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
<i>0 to 7 I.V. injection 25% mannitol, 467 m.Osm/1.73 M²</i>										
1 U	9 to 30	10.3	331.3	43.0	32.5	12.3	51.0	2.1	472	522
P	21		28.2	7.0	141.8	4.8	95.6	1.7 ¹	279	320
2 U	30 to 49	7.29	360.4	67.2	37.5	12.3	58.8	4.0	540	623
P	40		23.8	7.6	141.8	4.8	98.8	1.7 ¹	278	317
<i>50 to 57 I.V. injection 25% mannitol, 467 m.Osm/1.73 M²</i>										
3 U	58 to 80	17.7	327.2	27.0	30.0	6.0	47.6	2.1	440	486
P	71		49.2	7.0	134.3	5.0	90.8	1.7 ¹	288	327
4 U	80 to 99	13.7	325.3	27.5	37.5	8.8	52.8	3.2	455	539
P	90		40.2	7.6	137.3	5.6	94.0	1.7 ¹	286	326
<i>101 to 108 I.V. injection 25% mannitol, 467 m.Osm/1.73 M²</i>										
5 U	109 to 130	22.8	313.7	22.6	30.0	8.8	46.2	1.7	423	476
P	122		58.0	8.6	131.3	4.7	92.4	1.7 ¹	297	331
6 U	130 to 149	14.4	352.8	30.1	27.5	12.0	45.6	2.4	470	524
P	139		48.9	7.3	132.8	4.5	90.4	1.7 ¹	286	323

TABLE 1—Continued

D. W., 12 yrs., 26.4 kg., 1.06 M²

SUCROSE

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M ²	SUCROSE	UREA	Na	K	Cl	PO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
<i>0 to 42 I.V. injection 74.4% sucrose, 392 m.Osm/1.73 M²</i>										
1 U	47 to 68	14.8	284.0	28.2	46.0	4.1	46.4	1.9	411	501
P	55.2		41.3	5.6	136.3	4.1	98.8	1.3	287	320
2 U	68 to 88	10.6	308.2	48.4	43.0	7.2	47.8	2.4	457	556
P	77.8		33.2	5.5	137.5	4.2	101.6		282	314

90 to 112 I.V. injection 74.4% sucrose, 196 m.Osm/1.73 M²

3 U	114 to 141	15.3	292.1	34.4	44.0	8.0	47.8	2.2	428	522
P	129.5		41.9	5.9	140.0	4.1	101.6		294	328
4 U	141 to 161	10.6	327.2	40.0	43.5	14.2	47.4	3.0	475	590
P	151.2		34.0	6.2	142.5	4.5	102.4	1.5	291	326

L. D., 13 yrs., 38.9 kg., 1.31 M²

GLUCOSE

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M ²	GLUCOSE	UREA	Na	K	Cl	PO ₄	SO ₄	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. ²
P	-76		17.2	7.3	141.0	4.7	102.4	0.9		274	308
P-2 U	-61 to -38	0.86	468.9	275.3	180.0	54.0	127.5	7.7	14.4	1128	1149

*-24 to -23 glucose, oral, 366 m.Osm/1.73 M² in 70 cc. lemon juice**0 to 4 I.V. injection 50% glucose, 256 m.Osm/1.73 M² with 50 cc. saline*

1 U	5 to 26	9.04	348.2	61.4	82.5	14.2	58.4	1.4	2.1	568	618
P	15		38.0	7.3	132.0	5.6	100.0	0.9		284	312
2 U	26 to 46	7.79	353.8	71.1	92.5	13.5	70.0	1.8	2.5	605	660
P	37		38.7	8.8	134.0	5.5	100.0			287	318

52 to 56 I.V. injection 50% glucose, 476 m.Osm/1.73 M²

3 U	58 to 79	18.5	313.5	31.4	82.5	15.5	59.6	1.0	1.8	505	529
P	69		55.8	8.8	127.5	5.8	98.0			296	323

¹ Determination on pooled plasma.

² The value for total osmolality of the plasma was estimated as $2(\text{Na} + \text{K}) - 8 + (\text{urea})$. The formula is based on the assumption that the proteins are the only osmotically negligible ions and that urea is the only non-electrolyte. The total osmolality of the electrolytes may be expressed in terms of cation equivalents as $2(\text{Na} + \text{K}) + 1.5(\text{Ca} + \text{Mg}) - \text{Prot}$. Substitution of the values of 7 mEq/l for the sum of calcium and Mg, and of 18 for protein, leads to the previous expression.

xylose, sorbose and sorbitol. To conserve space, only two preliminary periods on the first two experiments and one in the case of a diabetic subject have been included. Also, all late post-loading periods and those with rapidly changing plasma levels during and immediately following injection of the loading solute have been omitted. The table contains information on the age, weight and surface area of the subjects and on the size of the dose administered. The tabulated data comprise the urine

volume, the concentrations in plasma and urine of the loading solute, as well as of urea, sodium, potassium, chloride, phosphate and, in most instances, sulfate. The sum of the solutes determined and the calculated total osmolarity are also given. The value for urine was estimated from the freezing point depression in a manner previously described (2), and that for plasma was calculated in the manner indicated in the footnote to the table.

It may be seen that the urine flows following loading increased in a widely varying manner from the pre-loading rate of about 0.5 ml/min. (3). The maximum rate was 22.8 ml/min. The smallest response was observed with urea and NaPAH and the greatest with mannitol. A comparison of the amounts administered indicates that the diuretic response bore no direct relation to the dose administered. The factors entering into relation have been discussed previously (2).

The total osmolarity of urine and plasma may be considered next. The calculated plasma osmolarity increased from a mean pre-loading value of 304 m. Osm/l. to as much as 370 m. Osm/l. in the case of urea, and to 362 m. Osm/l. in the case of sodium sulfate, increases of about 20 per cent above the basal level. The mean level for all diuretic periods was about 330 m. Osm/l. The total osmolarity of the urine decreased in all experiments during diuresis in a pattern discussed extensively elsewhere (1, 2).

The concentration of the loading solutes in the plasma increased by as much as 58 m. Osm/l. in the case of the mannitol experiment presented. In another experiment with mannitol a peak value of 62 m. Osm/l. was observed. Other large increases occurred with sucrose, glucose and the other sugars and sugar alcohols, substances which distribute themselves in a small volume of body fluid. A high plasma concentration was also observed in the urea experiment, following a very large dose. The increase in sodium was about 35 m. Osm/l. in the case of sodium sulfate, and was somewhat smaller with sodium chloride loading. With para-aminohippurate and creatinine the increase in the plasma was least marked, in the former case because of the small dose administered and a rapid rate of excretion, and in the latter because of a large distribution volume. The urine concentrations of the loading solutes varied considerably. In the case of urea the concentration remained constant at the pre-loading level despite a nine-fold increase of urine flow. It is noteworthy that the maximum urine concentration of all other nonelectrolytes tended to average about 330 m. Osm/l. The sodium concentration reached a maximum of nearly 400 m. Osm/l. with sodium sulfate, a value in excess of any previously reported. In the case of loading with sodium chloride a lower maximum of sodium, approximately 275 m. Osm/l. was reached, similar to values reported by McCance *et al* (4). Of the anions, chloride reached the highest urine concentration. In one experiment, not presented in the table, a concentration of 370 m. Osm/l. was reached at a rate of urine flow of approximately 2 cc/min. following a dose of sodium chloride of 590 m. Osm/1.73 M², a value in exact agreement with that reported by Davies *et al*. (5) and McCance (4). In the experiment presented, a lower value was measured at a much higher rate of urine flow. Para-aminohippurate and sulfate had lower maximum concentrations. It is of interest that the concentration of all loading solutes tended to decrease somewhat at the highest urine flows.

The urinary concentrations of the solutes other than the loading solute on the

whole decreased as expected with increasing urine flow. Several significant exceptions are noteworthy. In the experiments with sodium sulfate and sodium chloride the potassium concentration decreased much less than expected. This behavior of potassium following sodium administration is well known and needs no further emphasis. With sodium sulfate loading the chloride concentration decreased to lower levels than observed in any other experiment. This effect is similar to the phenomenon observed in dogs by Lotspeich (6). He found that the administration of chloride interfered with the reabsorption of sulfate. The results of the other diuretic experiments here presented would suggest that the relation between chloride and sulfate is of a specific nature rather than based on a non-specific osmotic effect.

A more complete presentation of the excretion of the electrolytes during osmotic diuresis is summarized in the 4 graphs constituting figure 1; the amounts of sodium, chloride, potassium and phosphate, in m. Osm/min. are plotted against the rate of urine flow for all 11 loading solutes. In figure 1A the sodium excretion, excluding loading experiments with sodium salts, is presented. Despite a great variability of the data, a clear cut relation between the rate of urine flow and that of sodium loss is apparent. Of the individual solutes only the apparently greater sodium loss during loading with glucose and creatinine is worth noting. Figure 1B, on the chloride excretion, from which the experiments on loading with sodium chloride have been omitted, presents a similar pattern. Noteworthy is the low excretion rate of chloride in the experiments on loading with sodium sulfate, a circumstance which has been commented on in the preceding section. A higher-than-average chloride loss is suggested with creatinine loading. In figure 1C are presented the potassium losses. Excepting the experiments on loading with sodium salts, all of which show increased excretion, they demonstrate independence to a large extent of the excretion of potassium from the rate of urine flow. The sweeping out of potassium by administration of sodium salts is illustrated clearly. A constant potassium clearance in water diuresis at rates of urine flow exceeding 1 cc/min. has been demonstrated by other workers (7). The relative constancy of the potassium excretion in osmotic diuresis other than that produced by sodium salts would suggest that an increase of osmolarity of the body fluids alone need not lead to marked losses of intracellular potassium as has been suggested on the basis of observations in dehydrated man and animals (8). Figure 1D presents the data on phosphate excretion. Their pattern on the whole is similar to that of the potassium values, although an upward trend with increasing urine flow is suggested. The outstanding exception refers to the experiments on loading with para-aminohippurate in which a distinct increase in the excretion of phosphate occurred. This finding raises interesting speculations with regard to a possible competitive relation between tubular processes concerned with para-aminohippurate excretion on the one hand and reabsorption of phosphate on the other. A constant rate of phosphate excretion has been observed repeatedly in water diuresis (9) but a moderate increase was found in dogs following injection of hypertonic salt solutions (10). The relative constancy of the excretion of potassium and phosphate over a wide range of urine flows is in marked contrast with the proportionality between excretion and flow for sodium and chloride. Among the various factors which may be invoked to explain this circumstance, the osmotic work involved may be cited. With increasing rates of flow the difference in concentrations

between urine and plasma for Na and Cl tends to become greater while it diminishes for K and phosphate. Consequently with increasing urine flow the renal work required to conserve the composition of the body fluids increases for the former and

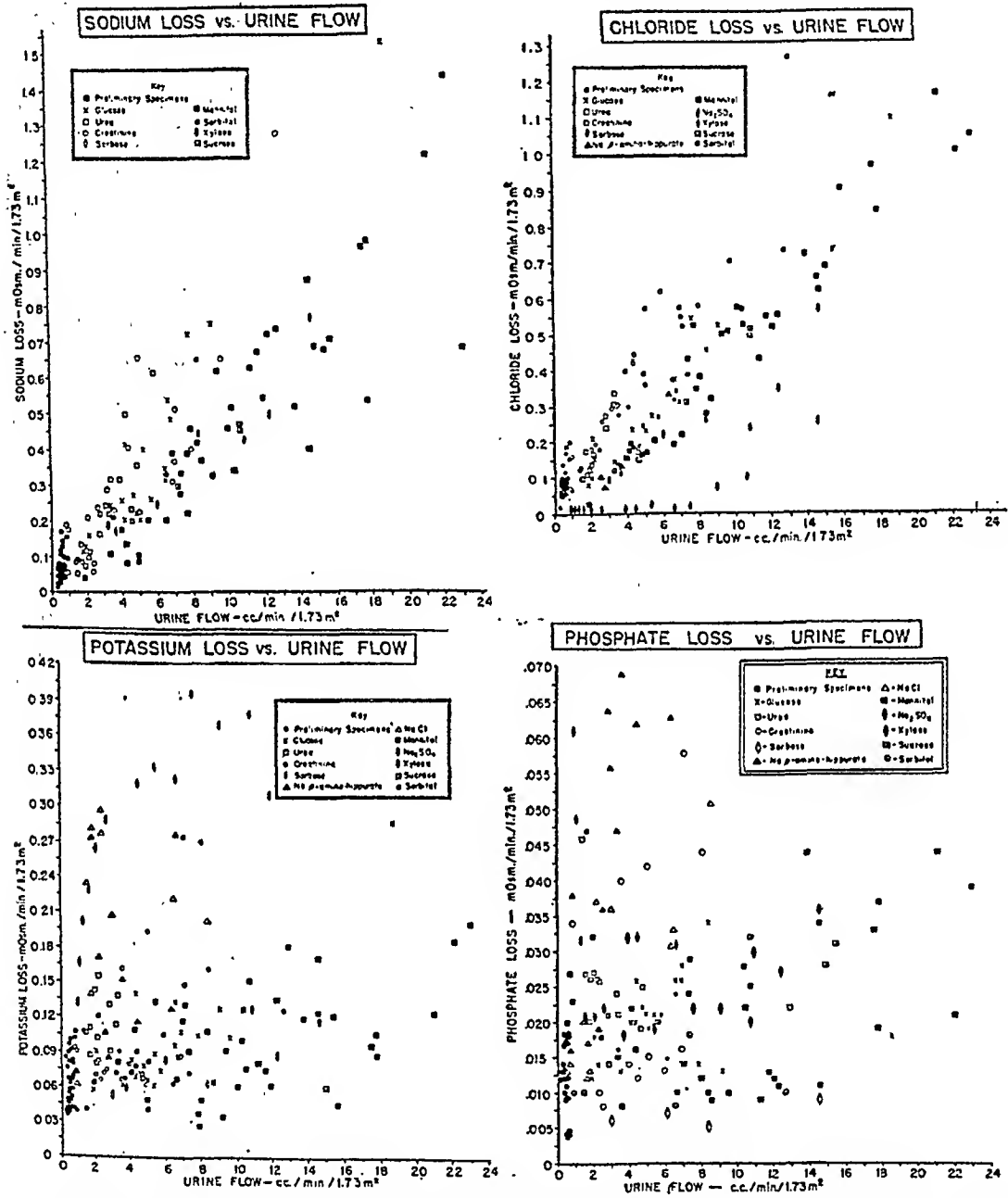


Fig. 1 A, B (upper left and right); C, D (lower left and right). URINARY LOSS OF SODIUM, CHLORIDE POTASSIUM AND PHOSPHATE DURING OSMOTIC DIURESIS. The electrolytes in m.Osm/min/1.73 M are plotted against the rate of urine flow in cc/min. All experiments with sodium salts are omitted from the sodium graph and those with sodium chloride from the chloride graph.

decreases for the latter ions. The urinary loss with diuresis of sodium and chloride may be a measure of the inefficiency of the renal regulation.

Renal Work in Osmotic Diuresis

This section of the paper deals with the ideal renal work involved in the process of urine formation under the conditions of osmotic diuresis. It may be well to state

clearly at the outset the limitations and uncertainties of the approach adopted. The general justification for the consideration of the renal work is the basic circumstance that energy is required to produce a given concentration difference between urine and plasma. In the absence of detailed knowledge concerning the exact pathway of urine formation and the metabolic efficiencies of the tubular processes engaged in the elaboration of urine, a minimal value for the renal work is given by the energy thermodynamically required to produce the observed concentration differences. The work is calculated on the assumption of an idealized single-stage process of urine formation conducted in an energetically reversible manner at 100 per cent efficiency. These basic premises are so far removed from the actual conditions of renal function as to raise considerable doubt concerning the biological significance of the calculated ideal work value. Some of the objections may be mentioned. 1) The concept of a single-stage process of urine formation is contrary to the known facts of renal physiology. 2) The assumption of complete efficiency is certainly invalid for any biological system. Actually, data in the literature (11-13) based on the oxygen consumption of the kidney, suggest an efficiency of only 1-2 per cent for the kidney. Furthermore it would be surprising that various types of renal processes, say those involving reabsorption of glucose and of electrolytes, should have the same energetic efficiency. 3) It is by no means certain that the basic assumption of the renal work as that of a chemical concentration cell for each individual solute is valid for all renal processes involving change in the osmolarity of the tubular fluid. In a later section reasons will be cited to doubt this premise for the work in the distal tubule. A number of other equally valid objections may be cited, all of which would suggest that there need be little or no connection between a calculated ideal work value and the actual expenditure of energy by the kidney. Nevertheless, with these reservations in mind, it appeared of interest to explore the possibility that the calculated work during osmotic diuresis would assume a pattern of biological significance and reflect in some manner the actual effective energy expenditure of the kidney, i.e. the portion available for the performance of external work. It appeared conceivable that during solute loading the useful energies of the kidney become engaged to a maximal extent. If so, a measure of the overall work capacity of the kidney would be obtained. Such a maximum would be defined experimentally by a failure to produce a greater work value by increase of either plasma level of loading solute, or urine flow, or urinary load. If such a maximum were discernible further questions would be in order: Is it the same or different for various solutes? How do the individual work values of different solutes contribute to the total? What conditions determine its occurrence? Further questions, not to be taken up here, refer to its possible clinical application. In the following section the ideal work will be presented first as an idealized single-stage process, both as to its total value and to its distribution among different solutes, and secondly subdivided as to proximal and distal urine formation based on assumptions to be discussed later.

Ideal total work. The ideal total work is presented for 30 periods on 8 loading solutes in the last column of table 2; and for all 11 solutes, plotted against the rate of urine flow, in figure 2. It may be seen that the work, after first rising with increasing flows, reaches a plateau at a value of about 4.0 cal/min. A more exact analysis of the relation between urine flow and work was attempted in several directions. For a

TABLE 2. IDEAL RENAL WORK DURING OSMOTIC DIURESIS¹
 Values are for periods listed in table 1; work values are expressed as cal/min/1.73 M²

C. B. UREA									
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	UREA	Na	K	Cl	PO ₄ ²	SO ₄ ²	GLUCOSE AND HCO ₃ ⁴	TOTAL
P-2	0.50	.53	.01	.06	.01	.02	.02	.01	.66
I.V. injection urea, 1385 m.Osm/1.73 M ²									
2	4.58	2.29	.13	.01	.09	0	0	.07	2.59
3	4.61	2.42	.17	.02	.11	.01	0	.07	2.80
G. P. CREATININE									
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	CREATININE	UREA	Na	K	Cl	PO ₄ ²	SO ₄ ²	TOTAL
P-1	0.66		.60	.01	.11	0	.04	.02	.79
I.V. injection creatinine, 144 m.Osm/1.73 M ²									
3	6.97	2.66	1.26	.19	.20	.02	.03	.01	4.48
4	3.51	1.63	.91	.08	.12	0	.02	.01	2.82
I.V. injection creatinine, 218 m.Osm/1.73 M ²									
5	7.96	2.54	.58	.24	.16	.02	.01	.01	3.68
6	4.97	2.27	.69	.16	.13	.02	.02	.01	3.38
W. H. NaCl									
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	Na	Cl	UREA	K	PO ₄ ²	SO ₄ ²	GLUCOSE AND HCO ₃ ⁴	TOTAL
I.V. injection NaCl, 1052 m.Osm/1.73 M ²									
1	8.36	.12	.23	.74	.13	.02	.01	.13	1.38
2	6.34	.07	.14	.44 ⁶	.16	.01	0	.10	.92
3	6.34	.05	.15	.45	.26	.01	0	.10	1.02
L. R. Na ₂ SO ₄									
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	SO ₄ ²	Na	UREA	K	Cl	PO ₄ ²	GLUCOSE AND HCO ₃ ⁴	TOTAL
I.V. injection Na ₂ SO ₄ , 758 m.Osm/1.73 M ²									
1	8.94	1.96	.40	.40	.23	.42	0	.14	3.55
2	5.31	1.16	.32	.42	.28	.29	.01	.08	2.56
I.V. injection Na ₂ SO ₄ , 506 m.Osm/1.73 M ²									
3	10.6	2.14	.43	.29	.20	.48	0	.16	3.70
4	7.45	1.58	.44	.43	.32	.45 ⁵	0	.11	3.33

TABLE 2.—Continued
NA P-AMINOHIPPURATE

A. R.

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	PAH	Na	UREA	K	Cl	PO ₄ ³	GLUCOSE AND HCO ₃ ⁴	TOTAL
<i>I.V. injection, NaPAH 113 m.Osm/1.73 M²</i>									
2	2.77	1.40	.04	.33	.04	.07	.08	.04	2.00
<i>I.V. injection, NaPAH 220 m.Osm/1.73 M²</i>									
4	4.35	1.54	.04	.43	.05	.07	.05	.07	2.25
5	3.52	1.50	.03	.45	.11	.05	.07	.05	2.26

A. R.

MANNITOL

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	MANNI- TOL	UREA	Na	K	Cl	PO ₄	GLUCOSE AND HCO ₃ ⁴	TOTAL
<i>I.V. injection mannitol, 467 m.Osm/1.73 M²</i>									
1	10.3	3.15	.25	.42	.02	.09	0	.16	4.09
2	7.29	2.82	.38	.27	.02	.05	.01	.11	3.66
<i>I.V. injection mannitol, 467 m.Osm/1.73 M²</i>									
3	17.7	3.53	.17	.71	0	.16	0	.27	4.84
4	13.7	3.19	.12	.47	.01	.11	0	.21	4.11
<i>I.V. injection mannitol, 467 m.Osm/1.73 M²</i>									
5	22.8	3.58	.10	.87	.02	.23	0	.35	5.15
6	14.4	3.37	.17	.60	.03	.14	0	.22	4.53

D. W.

SUCROSE

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	SUCROSE	UREA	Na	K	Cl	PO ₄ ³	GLUCOSE AND HCO ₃ ⁴	TOTAL
<i>I.V. injection sucrose, 392 m.Osm/1.73 M²</i>									
1	14.8	2.72	.20	.41	0	.18	0	.23	3.74
2	10.6	2.60	.39	.32	.01	.13	0	.16	3.61
<i>I.V. injection sucrose, 196 m.Osm/1.73 M²</i>									
3	15.3	2.84	.29	.47	.01	.19	0	.24	4.04
4	10.6	2.83	.25	.34	.04	.14	0	.16	3.76

TABLE 2.—Continued

L. D.

GLUCOSE

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M ²	GLUCOSE	UREA	Na	K	Cl	PO ₄ ³	SO ₄ ³	HCO ₃ ⁴	TOTAL
P-2	0.86	.57	.38	0	.04	0	.01	.01	.01	1.02

*Glucose, oral, 366 m.Osm/1.73 M²**I.V. injection glucose, 256 m.Osm/1.73 M²*

1	9.04	2.49	.42	.07	.02	.07	0	0	.11	3.18
2	7.79	2.17	.40	.04	.02	.03	0	0	.10	2.76

I.V. injection glucose, 476 m.Osm/1.73 M²

3	18.5	3.11	.18	.13	.06	.13	0	0	.23	3.84
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¹ The work was calculated according to equation 1. For this purpose the plasma concentrations of the loading solutes at the midpoint of the urine collection periods were determined by graphic interpolations from the slope of the disappearance curves. All plasma values were multiplied by 1.05 to give an assumed value for the concentration in the glomerular filtrate.

² Phosphate, plasma concentration assumed in m.Osm/l.; W. H., NaCl, 1.3; C. B., urea, 1.4; G. P., creatinine periods 3 and 4, 1.6, periods 6, 1.7; L. R., Na₂SO₄, periods 2 and 3, 1.2; A. R., NaPAH, periods 2 and 5, 1.3; D. W., sucrose, periods 2 and 3, 1.5; L. D., glucose, periods 2 and 3, 0.9.

³ Sulfate, plasma concentration assumed in all cases where not determined, 1.1 m.Osm/l.

⁴ The work is calculated on the assumption of a value for the sum of glucose and HCO₃ of 25 m.Osm/l in the plasma and zero in the urine. In the case of the glucose experiment, a plasma concentration of 20 m.Osm/l. was assumed for HCO₃ alone.

⁵ Plasma concentration assumed 102.0 m.Osm/l.

⁶ Plasma concentration assumed 4.8 m.Osm/l.

statistical estimate of the maximum work value 31 experimental periods with urine flows in excess of 10 cc/min. were considered. Three aberrantly low mannitol values observed during periods of rapid change of urine flow, were omitted. The remaining 28 periods yielded a mean value for work of 3.95 ± 0.09 cal/min., with a standard deviation of 0.49 cal/min. Calculation on this material of the linear regression of work on urine flow gave a small but significant regression coefficient of 0.064 ± 0.023 cal/min/cc. of urine flow. The probability that such a regression could have arisen by chance alone was 0.01. Tests for association were carried out on the mannitol experiments separately over a wider range of flows, in order to eliminate any effect arising from the multitude of loading solutes employed. They led to essentially identical results as those when all solutes were considered. Statistically, then, the rate of urine flow even in the range of high values exerted a slight effect on the magnitude of the work value. In the following discussion this subordinate effect for the most part will be disregarded.

All solutes are represented in the maximum range of work values excepting NaCl, urea and NaPAH. The fact that NaPAH fell short of the maximum is not surprising in view of the rather small amount administered. In the case of NaCl and urea, which were given in large dosage, a different explanation applies. Given a ceiling of urinary osmolarity, dependent on urinary flow (1), the initially high plasma

concentration of sodium and chloride limits the U/P ratio and therefore the possible work value below the maximum. In the case of urea the extensive tubular absorption in causing a rapid rise of the plasma concentration produces the same effect during loading.

The assumption of a maximum of work demands the determination of the independence of the work value not only from the rate of urine flow, but also from urinary

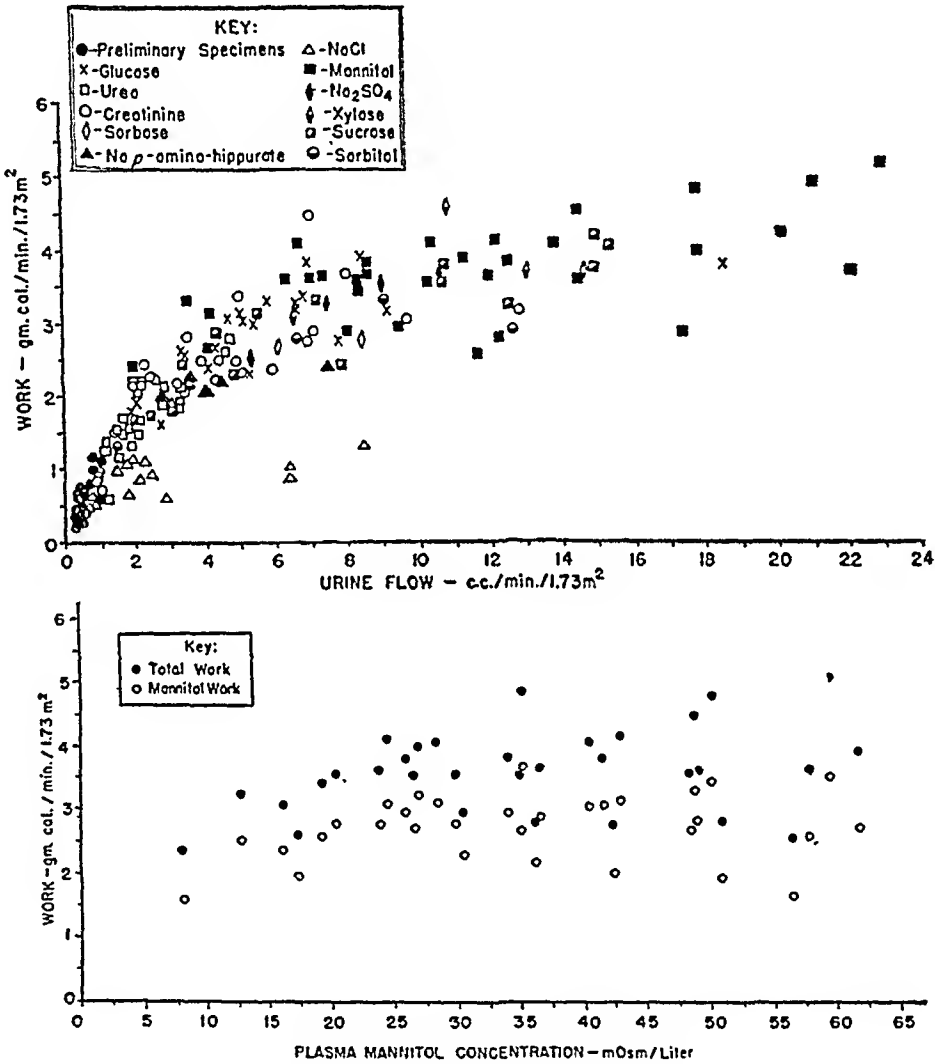


Fig. 2. IDEAL TOTAL WORK vs. urine flow

Fig. 3. IDEAL TOTAL AND MANNITOL WORK vs. plasma concentration of mannitol.

load and plasma level of loading solutes. A plot of work versus urinary load is in all respects similar to that versus urinary flow, shown in figure 2, and is therefore omitted. It also indicated on statistical analysis of the experimental periods at urine flows in excess of 10 cc/min. a slight but significant dependence of work on urinary load, with a regression coefficient of 0.19 ± 0.05 cal/min/unit load. The relation of work to the plasma level is exemplified by the data on mannitol loading summarized in figure 3. It contains a plot of total and of mannitol work versus plasma level of mannitol. An independence of the total work value from the plasma level over a

range from 20 to 62 m. Osm/l. is apparent. Statistical analysis of the data corroborates this impression. The linear regression of work on plasma level of mannitol yielded a non-significant coefficient of less than 0.005 cal/min/m. Osm/l. of plasma level. The probability of its occurrence by chance alone was 0.5. Thus, in this case, even a slight dependence of work on the plasma level, such as noticed previously with respect to urine flow and load, is absent.

In figure 4 is presented the work per cc. of urine elaborated, plotted versus the rate of urine flow. It is seen that this work decreased sharply at first and more slowly later. On the whole, the pattern of the graph is strongly reminiscent of that of the plot of urine osmolarity versus urine flow (2). Theoretically, once renal work reaches its maximum, $W/\text{cc.} \times \text{volume}$ becomes constant and a strict reciprocal relation between the work per cc. and urine flow obtains.

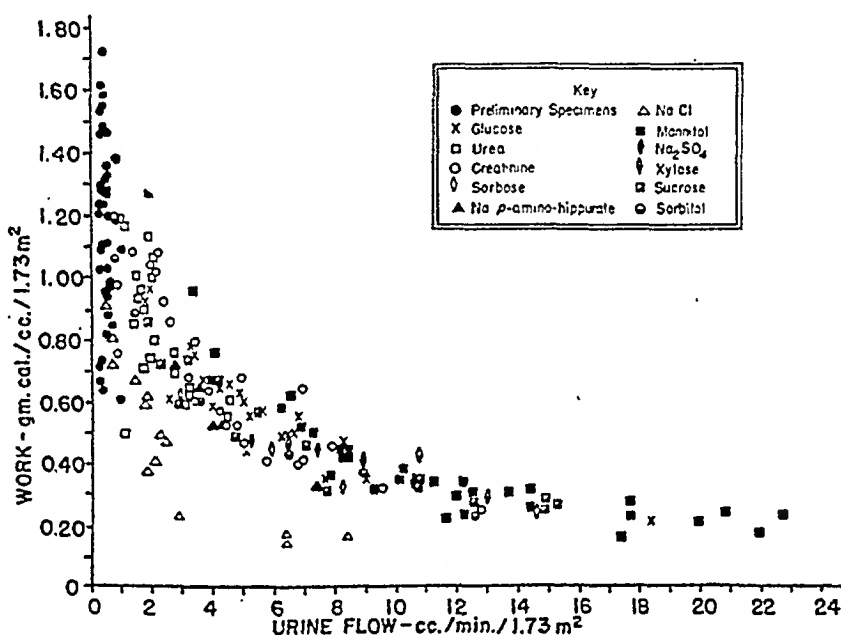


FIG. 4. WORK PER CC. OF URINE elaborated vs. rate of urine flow

An important question refers to the relation of the work to the duration of osmotic diuresis. *A priori*, 3 possibilities are apparent: 1) independence of the work value from the duration of diuresis; 2) exhaustion of the renal mechanism during continuous loading; and 3) a lag with increased efficiency during prolonged loading. Some of the relations observed are plotted in figure 5, which shows progressive changes in the relation between total work and flow during four representative experiments involving urea, sodium chloride, mannitol, and creatinine. Although there is no indication of any effect of repeated loading on the maximum work value, there is clear evidence of hysteresis, with the work during de-loading proceeding at a much higher level than during loading. The level during de-loading is close to the maximum.

The distribution of the work values among the different solutes may be considered next. Table 2 contains a compilation of the ideal work values during osmotic diuresis for the periods presented in table 1. Included are the individual values of the solutes determined, an assumed value for bicarbonate and glucose, and a calculated

total value. It is seen that in most instances, the loading solute accounted for the bulk of the renal work. Its contribution amounted to more than 85 per cent in the case of urea, and to more than 75 per cent in the mannitol, sucrose and glucose experiments. Similar high percentages were calculated for all loading solutes except for the sodium salts. With sodium para-aminohippurate, the anion alone accounted for about 70 per cent of the ideal work, while the sodium contribution was negligible. It was equally small in the sodium chloride experiment while the chloride contribution, although considerably greater, was only 0.23 cal/min. The highest value of sodium work was observed with sodium sulfate, where a value of 0.44 cal/min. was

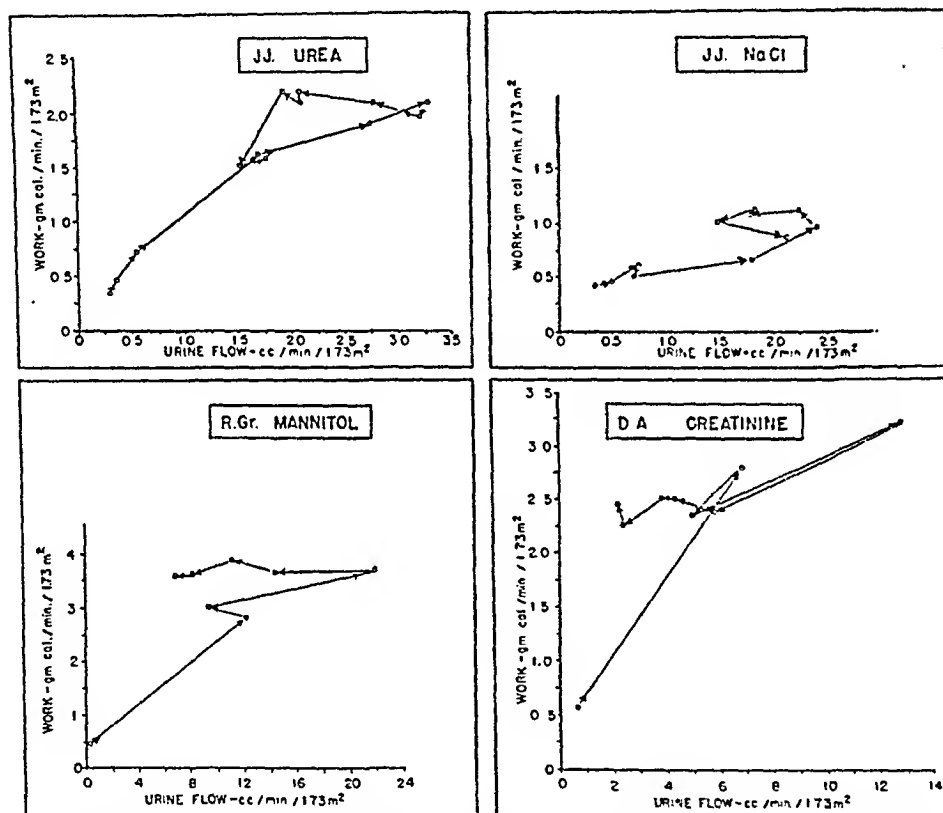


Fig. 5. SEQUENCE OF RELATIONS between work and urine flow during osmotic diuresis. Arrows indicate the order of the observations.

calculated, still only about 10 per cent of the total work. In that experiment the sulfate contribution accounted for approximately 50 per cent of the work. In experiments other than those involving sodium salts the sodium work tended to increase as the urine flow rose, while the reverse was true for potassium. Both of these trends are as expected, since with increasing flow the concentration difference between plasma and urine increases for sodium and decreases for potassium. Chloride on the whole followed the pattern of the sodium, and urea, phosphate and sulfate that of potassium. The principal exception refers to the potassium work during loading with sodium salts. Under these conditions its work value rose during diuresis. As a matter of fact the potassium contribution in one sodium chloride experiment accounted for 40 per cent of the total work. A relatively large value of the chloride work during

loading with sodium sulfate, a reflection of the phenomenon of depressed excretion discussed in the preceding section, is worthy of note. Also of interest is a high value of the urea work during one experiment with creatinine loading. But in two other experiments its contribution was not so great.

Osmotic work in proximal and distal tubule. Theoretical considerations. The calculations heretofore described have referred to the idealized direct process of urine formation from the glomerular filtrate. Actually there is good reason to believe that the concentration of a solute may change in opposing directions in different parts of the nephron. It is extremely unlikely that such changes are energetically reversible, i.e., that the energy expended by the cells in one portion of the tubule in producing a given concentration difference may be regained by those in another portion if the tubular fluid returns to a previous concentration. Therefore the work calculated for the different portions of the tubule, if the actual concentration changes in each portion were known, would be greater than that calculated on the assumption of a single-stage process. The knowledge of the actual stages of the process of urine formation is still too fragmentary to permit the calculation of the work for each segment of the tubule. However, one over-all division of the process of urine formation has become generally accepted and may be profitably discussed. It is assumed that the process of water reabsorption proceeds in two stages: The first in the proximal tubule, at a constant osmolarity, isotonic with plasma, but with a changing distribution of solutes; and the second distally, with the production of a hypertonic urine (14). Previously (1, 2) a dependence of urine flow and osmolarity on solute load which was independent of the composition of the urine has been demonstrated. The character of this relation could be explained on the assumption of a constancy of the solute load in the distal tubule, with reabsorption of water alone taking place. The process of water reabsorption could be expressed in terms of load as follows:

$$R_{pr} = GFR - \frac{\text{Load}}{O_p} \quad \text{and} \quad R_d = \frac{\text{Load}}{O_p} - V \quad (2)$$

where R_{pr} and R_d refer to the amounts of water reabsorbed in the proximal and distal tubules respectively, O_p is the total osmolarity of plasma, assumed to be equal to that of the fluid in the proximal tubule, GFR is the amount of glomerular filtrate and V of urine. On the basis of these relationships calculation of the concentration of the solutes and the volume of fluid at the dividing point of proximal and distal tubule, and therefore a separate consideration of the work in the proximal and the distal tubule, become feasible. The concentration of a solute, as it leaves the proximal tubule, U_{pr} , is given as

$$U_{pr} = \frac{U \times O_p}{O_u} \quad (3)$$

where U refers to concentration of the solute in the urine, O_u is the osmolarity of urine and O_p that of the fluid in the proximal tubule, assumed to be isotonic with plasma. The volume of the fluid as it leaves the proximal tubule, V_{pr} , is given as

$$V_{pr} = \frac{V \times O_u}{O_p} \quad (4)$$

where V is the volume of the urine and the other symbols have the same meaning as before. With these data on hand the work in the proximal tubule for a given solute may be calculated on the basis of *equation 1*. The total work in the proximal tubule is given by the sum of the work values for all individual solutes.

Two alternative processes may be considered for the work in the distal tubule: 1) a process similar in nature to that in the proximal tubule. In essence, this would mean that in the distal tubule, as proximally, work is done on each solute independently, varying for each constituent with its specific concentration ratio. The total work, varying according to the composition of the solute load, is given by summation of the individual work values. The situation would be comparable to the calculation of the energy output of a series of concentration cells of different chemical composition. This assumption may be designated as the 'chemical' theory of distal work. 2) It is not the chemical composition but only the osmolarity of urine and plasma that determine the necessary work. The situation would be analogous to the work process across a semi-permeable membrane, which is permeable to water alone but not to any solute particle. The energy of such a process would be given by the same equation as previously employed, save that the total osmolarities of plasma and urine are used instead of the individual concentrations of the several solutes. The factors determining total work in the distal tubule would then be the ratio of the urine and plasma osmolarities and the urine flow, independent of the chemical composition of the solute load. This hypothesis may be called the 'osmotic' theory for the work in the distal tubule. In general the total work value calculated in this way will be smaller than that based on the 'chemical' theory.

At present a decision between these two theories is not at hand. But two circumstances which are difficult to explain by the 'chemical' theory, but are predicted by the 'osmotic' theory, are apparent: 1) the existence of a maximal osmotic ceiling for urine at minimal flows, which is independent of its composition; and 2) the dependence during osmotic diuresis of urine flow and osmolarity on load alone, independent of the nature of the loading solute.

One implication of the 'osmotic' theory may be mentioned. It would suggest that water alone moves across the distal tubular wall, either in the process of reabsorption for the production of hypertonic urine, or secretion during water diuresis. The function of the antidiuretic hormone may well be linked up with the energetics of this process.

In figure 6 is depicted a graph of the relation of work and load in the distal tubule on the basis of the 'osmotic' theory. It is constructed on the assumption of a constant value of 0.33 Osm/l. for the osmolarity of the fluid in the proximal tubule. It may be seen that the distal tubular work reaches a maximum at a load of about 4 m. Osm/min., corresponding to flows of 8 cc/min. With further increase in load and flooding of the distal tubule the work delivered actually decreases. The total work in the distal tubule even at its maximum represents only a small fraction, approximately 12 per cent, of the proximal work.

Application to present experiments. It appears of great interest to apply the concepts discussed to the experimental material at hand.

Before engaging in a detailed scrutiny of the data one can predict that this form

of calculation will result in a decreased work contribution for those solutes that are little or not reabsorbed in the tubule. On the other hand the work will increase for

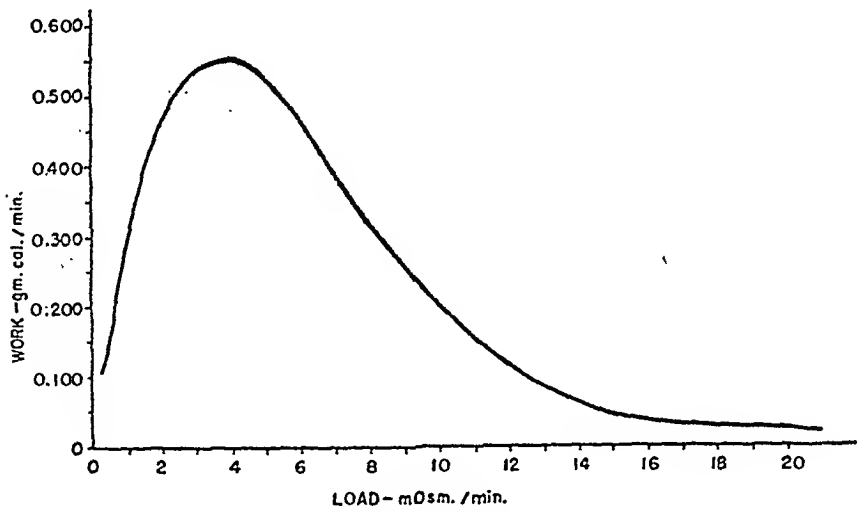


Fig. 6. WORK VS. LOAD IN THE DISTAL TUBULE, calculated on the assumption of an osmolarity of 0.33 Osm/l. for the fluid leaving the proximal tubule.

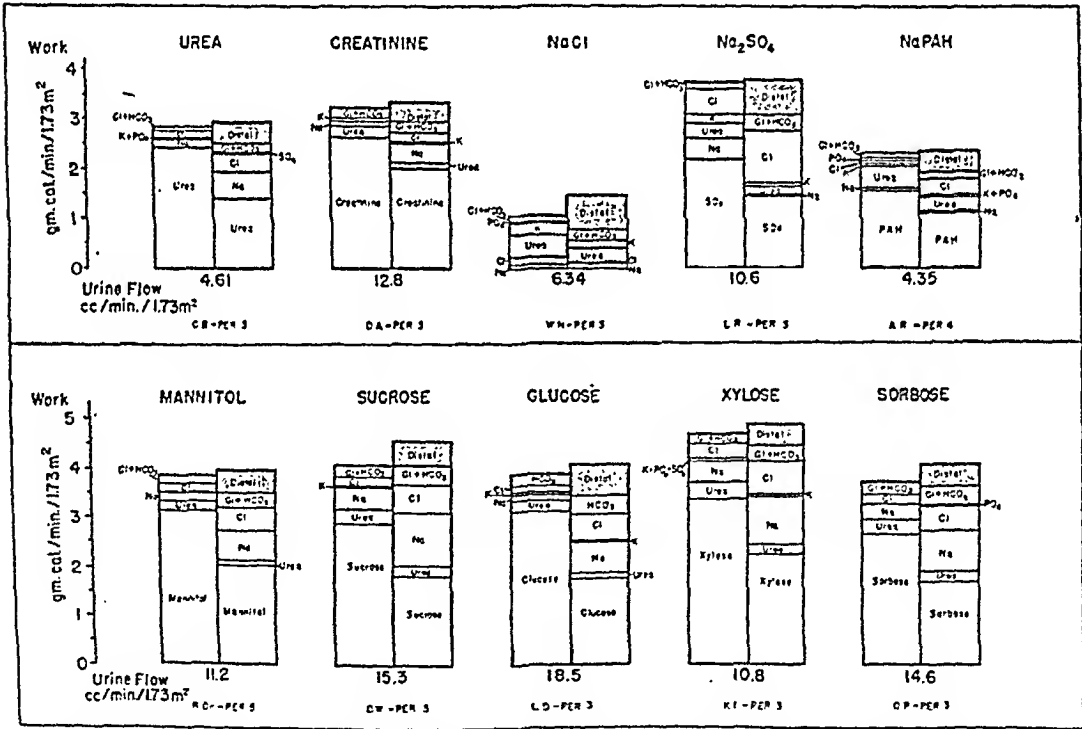


FIG. 7. COMPARISON OF IDEAL TOTAL WORK with proximal and distal work for 10 loading solutes. The work values for the individual solutes are graphed in an additive manner in bar diagrams. The distal work is indicated by a shaded area. Periods of maximal work were chosen for the comparisons.

the solutes that tend to be reabsorbed extensively. In figure 7 are presented illustrative comparisons between the ideal total work, and the proximal and distal work for 10 of the loading solutes, sorbose alone having been omitted. Periods of greatest work for each loading solute were chosen for the presentation.

For the osmotic work in the proximal tubule, the volume and solute concen-

trations of the fluid at the junction of the proximal and distal segments of the tubule were calculated by *equations 3 and 4*. For the calculation the value O_p , the osmolarity of the fluid in the proximal tubule, was calculated for each period as described in the footnote of table 1. The method of calculating the urine osmolarity, O_u , has been described previously (2). The value of V_{pr} , the volume of the fluid as it leaves the proximal tubule, and U_{pr} , the concentration of a solute in the fluid, were substituted for V and U respectively in *equation 1*. The total work in the proximal tubule was the sum of the works for each solute.

For the calculation of the osmotic work in the distal tubule the osmolarity of urine, O_u , and the osmolarity of plasma, O_p , were substituted for U and P in *equation 1*.

The outstanding points in the comparison are as follows: 1) the total work value on the whole is the same, whether it is calculated as a single-stage chemical process (ideal total work) or is subdivided into proximal and distal contributions; 2) the

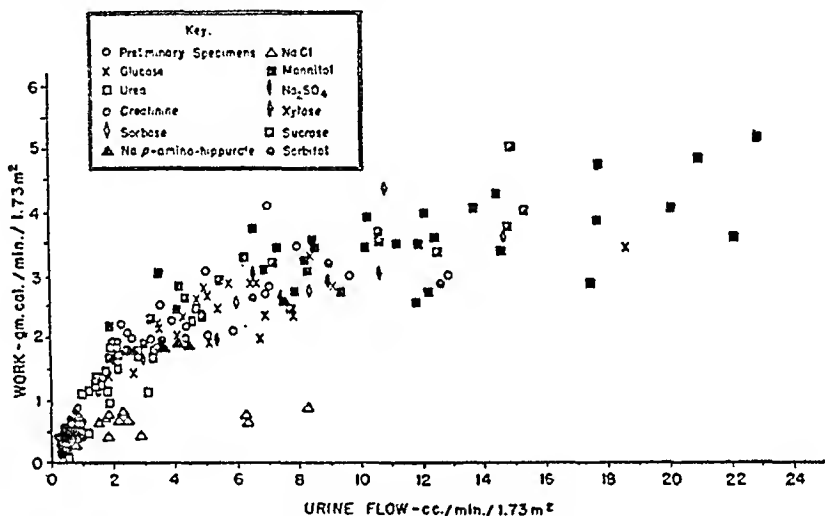


Fig. 8. PROXIMAL WORK vs. urine flow

distal work is always a small fraction of the total which reaches a maximum at urine flows of about 8 cc. per minute; 3) although the proximal work value approximates the ideal total work, the distribution of the work among individual solutes differs considerably. The variations are in the predicted direction. In all loading experiments not involving sodium salts, the sodium and the chloride work assumes a prominent place, while the work of the loading solute decreases.

In figure 8 a plot of the proximal work versus urine flow for all solutes is presented. It is evident that its pattern is entirely similar to that of figure 2 which depicts the relation of the ideal total work versus urine flow. A maximum of the same magnitude is apparent. The conclusion appears justified that the ideal total work in effect represents the proximal work value.

DISCUSSION

The following discussion will deal with 2 items: 1) a schematic presentation of the inter-connection between the urinary flow-load relation on the one hand, and on the other the renal work; and 2) the relations between the calculated work and the actual energy metabolism of the kidney.

1) *Theoretical work for a single solute.* The general equation for renal work (1) may be expressed more suitably for this discussion as

$$W = RT \text{ load } (\ln U/P + P/U - 1) \quad (5)$$

where W is the work, V the urinary volume, U the urinary and P the plasma concentration of a given solute, and load is equal to $U \times V$, has 3, *a priori*, independent variables. It contains no inherent limitations for the magnitude of any of the variables, or the work value. The experimental fact that urine flow and urinary osmolarity are not independent of each other, but are a function of load, may be used to reduce the number of variables to 2, if the simplifying assumption is made that a single solute accounts for the entire osmolarity of urine. Then U for man may be expressed (2) in terms of load as

$$U = 0.847 e^{-0.21 \text{ load}} + 0.33. \quad (6)$$

Substitution of this expression for U in equation 2 permits one to define work in terms of load and plasma concentration alone. Actually, even under conditions of maximal work, as shown in the experimental part, the loading solute accounts for only about 80 per cent of the urine osmolarity. The simplification employed qualifies the quantitative applicability of the derivations but does not modify to an important extent the significance of the patterns.

Another experimental datum, the existence of a biological maximum of work of 4.0 cal/min. for man, serves to delimit the biologically possible magnitude of the work value.

Thus it is possible to give a schematic portrayal of the inter-dependence of W , load, and P in the biologic range, incorporating the determined flow-load relations on the basis of the assumption of a single urinary solute. A complete presentation would require a three-dimensional grid. Here the inter-relations are presented in the form of 3 graphs, in each of which 2 of the factors are plotted as continuous variables on the ordinates, and the third one is fixed at a series of values.

In figure 9 work is plotted against load for a series of different assumed plasma levels of solute. Certain values of urine flow corresponding to the given loads are also indicated on the abscissa. A dotted line indicates the position of the approximate biological maximum of renal work for man. It may be seen that for low plasma levels the work rises steeply with increasing load. As the plasma level increases the work curves describe increasingly flat trajectories to reach the biological maximum at increasingly high loads. Finally at plasma levels between 100 and 150 the biological maximum is unattainable within the limits of biologically possible urine flows. It is evident that once maximum work has been reached further loading will result only in a horizontal displacement of the work value, although plasma levels and urine loads increase. It is also of interest to view the values for plasma and urine which are coincident with maximum work from the standpoint of clearances. It may be seen that for a plasma level of 1 m. Osm/l. maximum work is reached at a load of 1 m. Osm/min. This corresponds to a clearance of 1000 cc. of plasma/min., obviously an impossible value. With a clearance of 100 cc., i.e., approximately at the level of glomerular filtration, maximum work is reached for a

plasma level of 40 with a load of 4 m. Osm/min. These figures are of the same order of magnitude as has been actually observed under conditions of loading in mannitol experiments.

In figure 10 work is plotted versus plasma level for different loads. The plasma values are arranged in a logarithmic manner. This graph brings out the fall in work with increasing plasma levels and constant load, an aspect which has been stressed by Newburgh (15). It also illuminates the circumstance that for high plasma levels increase of load leads to only minor change in the work value. It is clear that the biological maximum is unattainable for high plasma values.

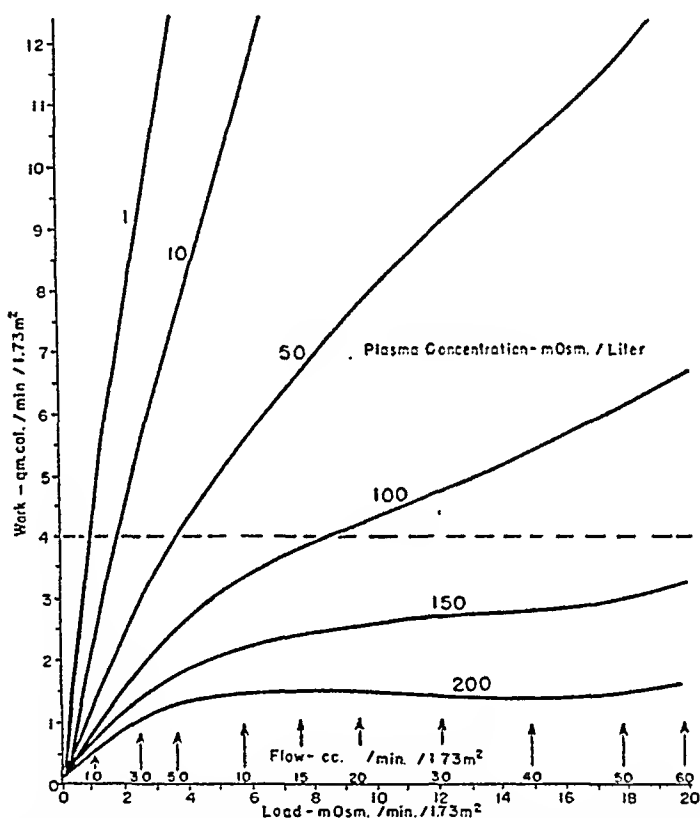


Fig. 9. WORK VS. LOAD for different plasma concentrations of the loading solute, calculated on the assumption of a single urinary solute.

In figure 11 is presented the third aspect of the relations. Load is plotted versus plasma level for a series of work values. Both variables are plotted in a logarithmic manner. This graph brings out the relationship between plasma and urine for constant work. The steep final portions of the graphs deserve emphasis. They illustrate the increasing inefficiency of increasing loads to produce increased work at high plasma values.

The theoretical impossibility to reach the biological maximum of renal work at high plasma levels within the known limits of the load-flow relations has interesting implications. It serves to explain the inability to produce maximal work by loading with sodium or chloride, solutes normally occurring in high concentrations in plasma. It indicates a similar limitation for urea, which because of its tubular reabsorption,

and low clearance, rapidly reaches high values in plasma during loading. Finally it applies to pathologic conditions, where a given work value may be maintained, with increasing loads cleared, at the expense of high plasma concentrations.

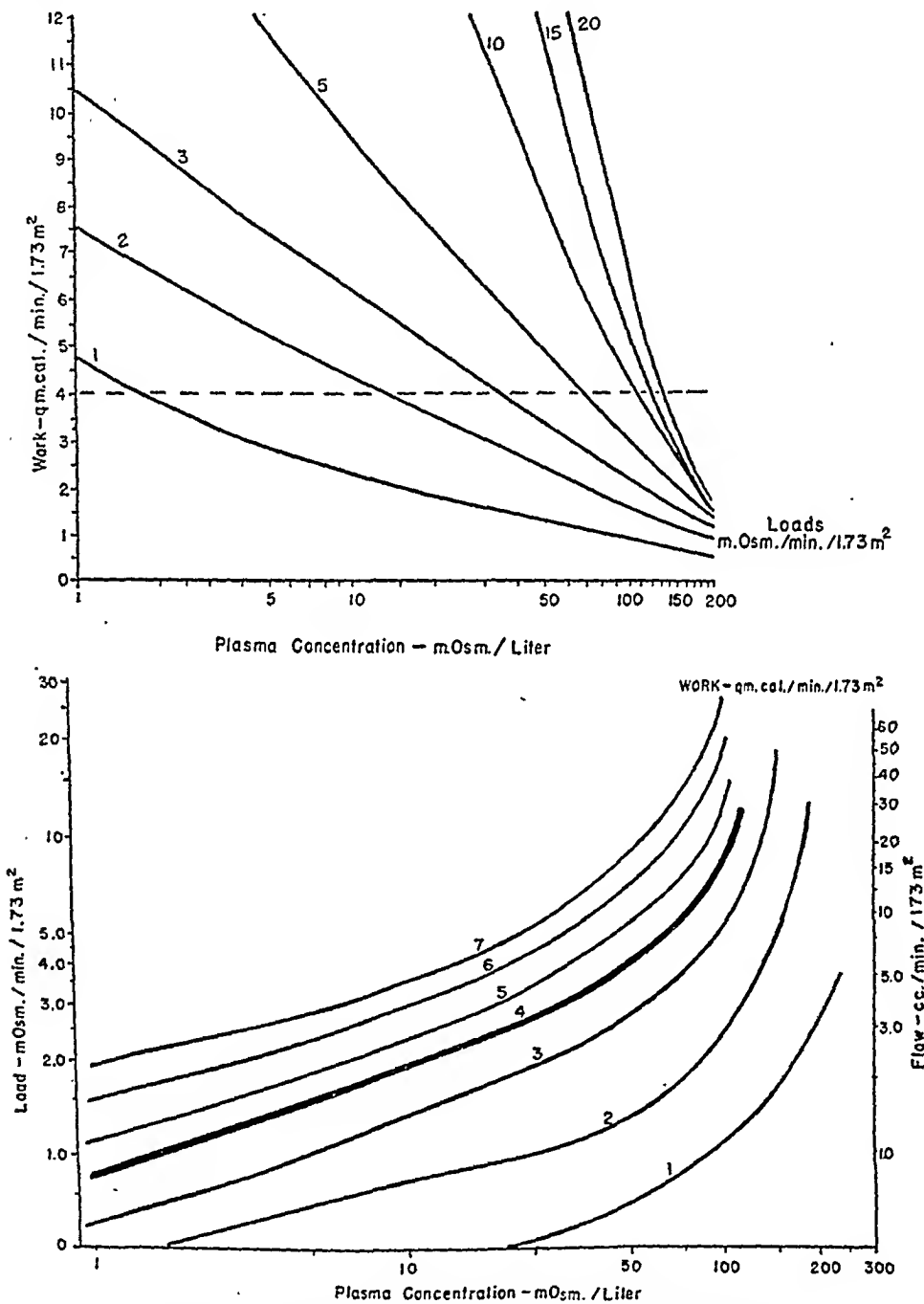


Fig. 10. WORK VS. PLASMA CONCENTRATION for different loads

Fig. 11. LOAD VS. PLASMA CONCENTRATION for different work values

2) *Calculated work and energy metabolism.* The oxygen consumption of the kidneys of man under 'resting' conditions has been estimated by Bradley and Halperin (6) on a small group of subjects to be about 10 cc/min. with a range of ± 4.0 cc. This corresponds to about 0.03 cc. of oxygen per gm. of tissue, if an average weight of 300

gm. is assumed for the kidneys of man. In dogs, an average value of 0.07–0.08 cc. per gm. (12, 17) or higher (18, 19) and an even greater variation has been reported. Assuming a respiratory quotient of 1.0 so that 1 cc. of oxygen corresponds to 5.0 cal., and as a minimum figure for the oxygen consumption, the resting value of 10 cc/min., a total energy of 50 cal/min. would be available to the kidneys of man. It has been shown that the thermodynamic work of the hydropenic kidney under resting conditions accounts for about 0.6 cal/min. (3), a little more than 1 per cent of the total energy available. This proportion is of the same order of magnitude as has been measured directly in animals (11, 12) and estimated indirectly for man (20). During osmotic diuresis, the thermodynamic work rises to an average maximum level of 4.0 cal/min., accounting at the most for 8 per cent of the total energy available, and probably for less. An increase in efficiency during diuresis, i.e., a relatively greater increase in the work compared with the oxygen consumption has previously been reported for the isolated kidney of dogs (13). The effect of osmotic diuresis on the oxygen consumption has been variously reported. Whereas early workers described an increase during diuresis produced by either urea or sodium sulfate (21), later observers failed to find any correlation between urine flow or thermodynamic work and oxygen consumption (12, 13, 17, 22). On the other hand a positive correlation between oxygen consumption and blood flow was described (12, 17). Van Slyke *et al* (17) suggested that both blood flow and oxygen consumption increased in response to the metabolic demands of the kidney and concluded: "The overwhelmingly greater part of the energy must be utilized by the kidney for its own internal cellular processes not related to the external work which the organ is performing."

Although the conditions of the experiments cited differed considerably from those obtaining in our study, since all previous workers secured a copious urine flow by hydration of their subjects or experimental animals, their main conclusion probably applies, namely that thermodynamic work accounts for only a small fraction of the total energy, and that there is little relation between the external work of the kidney and its metabolic demands. On the other hand the existence of a reproducible maximum of thermodynamic work suggests that the calculated values reflect some aspect of the energy metabolism of the kidney, perhaps its actual effective external energy expenditure. Whether this portion of the renal energy is so small as to be obscured by other metabolic needs of the kidney; or whether one is dealing with a diversion of the energies from internal to external use, so that their sum remains constant, cannot be decided at present. The increase in efficiency during diuresis, reported by Eggleton *et al*. (13) perhaps favors the latter hypothesis.

Accepting the premise of the biologic validity of the calculated data, certain implications of a maximum of renal work should be pointed out. In particular, the circumstance that the same maximum was calculated for a variety of solutes, appears surprising in view of the presumably widely differing renal mechanisms engaged.

Considering first the non-electrolytes, the equality of maximal work for glucose, which is reabsorbed to a significant extent in the tubule, and sucrose, which is not, would suggest that the tubular reabsorption process contributes only a minor share to the total work. The work imposed by the fraction escaping reabsorption over-

shadows it by far, and may be quite similar among the various sugars and sugar alcohols. Such an explanation does not satisfy in the case of sodium sulfate loading. A similarity in the efficiency of widely varying renal mechanisms may be proposed, or the existence of a common energy pool from which all renal processes draw. Given a fixed total value of osmotic work capacity the distribution of the renal work among the several processes would then vary according to the type of loading solute. The existence of such a common energy pool, for which various renal mechanisms compete, would betray itself by the fact that although the total work value remains constant the distribution of the work among different solutes may vary with the urine flow. With increasing flow one would expect the contribution of the loading solute to diminish and that of the sodium and chloride to increase. The experimental observations are in agreement with this concept. A further consideration may be advanced. One might reason that under the circumstances of hydropenia considered, with water as the over-all limiting factor the bulk of the work consisted in abstracting water from the loading solutes and preserving the body electrolytes. The work considered in the present experiments may then have neglected other external functions of the kidney.

Another question arises as to whether the maximum found in the hydropenic state describes the over-all osmotic work capacity of the kidney under all conditions. An obvious extension of the inquiry should encompass a study of water diuresis and of the rôle of the anti-diuretic hormone.

SUMMARY

The excretion of solutes and the renal work under conditions of osmotic diuresis in man has been presented. Eleven loading solutes were administered in amounts of 500–2000 m. Osm/1.73 M² body surface. They were: glucose, sucrose, mannitol, sorbitol, sorbose, xylose, urea, creatinine, sodium para-aminohippurate, sodium sulfate and sodium chloride.

During loading the urine flow increased widely up to 22.8 ml/min. The plasma osmolarity averaged 330 ± 30 m. Osm/l., while the urine osmolarity decreased. The concentration of the loading solute in plasma rose as high as approximately 60 m. Osm/l. in the case of mannitol. In the urine it averaged 330 m. Osm/l. for most loading solutes with wide individual variations. As the urine flow rose the excretion of sodium and chloride rose proportionately, while that of potassium and phosphate tended to remain constant. Sodium salts produced an increased potassium loss, while sulfate decreased the chloride loss.

The calculated ideal osmotic work rose during loading to a maximum of about 4.0 cal/min., a value which was not increased by further augmentation of urine flow or load or plasma level of the loading solute. The same maximum value was reached with all loading solutes studied excepting NaCl, NaPAH and urea. NaPAH was not administered in adequate amounts, but in the case of urea and NaCl, theoretical reasons exist for the failure to reach maximum work.

A subdivision of the renal work in proximal and distal portions, based on the observed flow-load relations of urine has been attempted. It is suggested, that while the proximal tubular work is essentially 'chemical' in nature, depending on the

concentration in plasma and urine of individual solutes, that in the distal tubule is 'osmotic,' depending on the total osmolarity of plasma and urine. The proximal work is closely similar in magnitude to the ideal total work, although the distribution of the work values among the individual solutes differs widely. The distal work is usually a small component of the total work. The relations among the renal work, the plasma concentration and the urinary load under the simplifying assumption of a single urinary solute have been discussed and graphically presented.

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INULIN SPACE AS A MEASURE OF EXTRACELLULAR FLUID

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THE measurement of the extracellular volume must be made with a substance which is completely distributed in that space but excluded from the intracellular compartment. Ideally this substance should also fulfill the following conditions: 1) fairly rapid and uniform distribution; 2) no formation or destruction in the organism; 3) negligible osmotic effect; 4) slow or measurable elimination from the body; 5) no toxicity, and 6) accurate and easy determination.

The first attempt to measure the extracellular space was made visually in frozen preparations of muscle (1) and the value obtained was 15 per cent of the total weight. The fact that the total quantity of chloride found in muscle, if distributed in that histological space, would be in the same concentration as in the plasma, led to the conclusion that all the chloride was extracellular (2). This assumption was extended to include the whole body, and chloride and consequently sodium were considered to be limited to extracellular distribution (3-9), although it has been recognized that sodium and chloride enter the cells to a variable degree (5, 7, 8, 10-13). Methods using these electrolytes will give volumes of distribution which are variably larger than the actual extracellular space. This conclusion can be extended to bromide which is distributed in the same volume as chloride (14, 15). Sodium thiocyanate, one of the substances most widely used for this purpose, also enters the cells (16, 9, 17, 18). When the above substances were used simultaneously in the dog, the values obtained were: thiocyanate, 35.6 per cent of body weight; radioactive sodium, 27.6 per cent; and radioactive chloride, 24.7 per cent (9).

To avoid the disadvantage of the entrance of electrolytes into the intracellular space other substances were sought to which the cell membrane was impermeable. Attention was focused on the carbohydrates, sucrose (19), mannitol (20, 21), and inulin (13, 22). Sucrose was used despite the disadvantages of a rapid excretion and an incomplete urinary recovery in the dog (23). The recovery of mannitol in urine is also incomplete (24, 25), indicating some utilization.

Inulin has several advantages over any of the above substances. It is not an electrolyte, is lipid insoluble and has a large molecular weight, all circumstances that reduce the probability of its permeating the cellular membrane. It does not penetrate the erythrocyte (26), diffuse through the normal renal tubule (26, 27), or undergo concentration by the liver cells (22, 28). The circumstance that it is rapidly and quantitatively recovered in the urine argues against its being metabolized to any appreciable degree or stored in any tissue (24, 29, 30). Furthermore, it is physiologically inert and exerts negligible osmotic pressure (29, 31). It has the disadvantage of being rapidly excreted by glomerular filtration, which prevents uniform distribution throughout the extracellular space after a single injection. This fact has limited its use, when given by the customary methods, to nephrectomized animals (13, 22), in which the volume of distribution is significantly smaller than the volume of distribution of chloride or thiocyanate.

An equilibration method which permits the use of inulin for the measurement of extracellular fluid in normal animals and man has been reported in a preliminary

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paper by the present authors (30). The procedure was devised to overcome the rapid excretion of inulin. The purpose of this paper is to report the complete results obtained with this method and the modifications introduced to minimize the errors arising in its practical application.

METHODS

Inulin space determinations were made on normal trained, unanesthetized dogs. The procedure depends on the maintenance of a steady infusion which compensates for excretion and maintains the plasma level constant until a uniform concentration exists throughout the extracellular space. Extracellular equilibrium is more rapidly attained if the infusion is preceded by a priming dose, calculated for each dog according to the desired plasma level and expected volume of distribution. All the experiments have been done with an infusion rate less than 1 cc. per minute so that the extracellular space is not significantly augmented. The constancy of the infusion afforded by the mercury drip method has proved to be adequate and the accuracy of the measurement does not increase with more elaborate means of maintaining constant infusions.

Once equilibrium is established, a blood sample is withdrawn, the bladder is emptied by catheter and rinsed and the infusion simultaneously discontinued. The urine is then collected until the total amount of inulin contained in the body has been excreted, 5 hours in the dog, 12 hours in man (30). The quantity of inulin recovered in milligrams, divided by the plasma concentration in mg/cc. equals the volume of distribution in cubic centimeters.

Prior to the inulin injection a control blood and timed urine sample are obtained for the determination of the corresponding blank corrections (inulinoid plasma blank in mg/cc. and urine inulinoid blank in mg/minute).

Thiocyanate and radioactive sodium (Na^{24}) spaces were determined by the single injection method. Blood samples for the determination of these substances were withdrawn after 1 hour for thiocyanate and after 3 hours for Na^{24} . Chemical analyses were made by the method of Harrison (32) for inulin, and Crandall and Anderson (16) for thiocyanate. Radioactive sodium was measured with a Geiger-Müller counter.

RESULTS

Equilibration Time. The length of infusion necessary to insure adequate and uniform distribution of inulin throughout the extracellular space has been determined experimentally. We consider that equilibrium is reached when any further prolongation of the infusion will not increase the inulin volume.

Determinations were repeatedly performed in the same animal with varying durations of infusion. Twenty-one experiments in 5 normal dogs show that equilibrium is obtained within 2 hours (table 1, *experiments 1-21*). In *dog 1* the infusion was maintained for 2, 3.3, 4.5, and 12 hours respectively with no significant change in the volume of distribution.

Three normal dogs were nephrectomized bilaterally and given a single injection of inulin. The plasma concentration was determined every 20 to 30 minutes during

the first 2 hours, and every hour thereafter. The observations were prolonged for 12 hours. The time necessary for uniform distribution varied from 1 to 2 hours (fig. 1), confirming the observations made in the normal dogs. Thiocyanate, injected simultaneously, reached a constant plasma concentration in 40 to 90 minutes.

As the inulin and thiocyanate spaces were followed, it became apparent that after equilibrium had been established and maintained for about 6 hours, both spaces began to increase (fig. 1). It is probable that this represents a terminal pathological shift of intracellular water in the nephrectomized animal. The results obtained in

TABLE 1. VOLUMES OF DISTRIBUTION OF INULIN, THIOCYANATE AND RADIOACTIVE SODIUM IN DOGS

EXPER.	DOG	BODY WT.	INULIN					VOLUME OF DISTRIBUTION (% BODY WT.)		
			Duration of infusion	Inulin recovered	Urine flow	Delay time correction	Inulin space	Inulin	Thio- cyanate	Sodium ²⁴
		kg.	hr.	mg.	cc/min.	% amt. recovered	l.			
1	1	17.0	2.0	662	.5	16.4	3.240	19.0	35.5	
2	1	16.4	3.3	635	.8	14.2	3.260	19.8	34.3	31.4
3	1	19.0	4.5	1011	3.5	2.8	3.560	18.7		27.0
4	1	19.0	12.0	967	3.6	2.8	3.540	18.7	34.5	27.5
5	2	18.0	2.0	362	1.4	6.9	3.510	19.5	32.0	31.0
6	2	17.1	2.5	251	3.8	4.0	3.000	17.6		32.4
7	2	17.0	3.3	231	2.2	7.0	3.120	18.4	29.9	
8	2	19.3	3.5	784	2.2	4.7	3.830	19.8		
9	2	16.8	4.0	255	1.3	6.7	2.940	17.5	34.4	
10	3	15.0	3.3	202	3.7	5.0	3.040	20.3	32.5	26.7
11	3	15.0	3.8	167	2.2	6.0	3.020	20.1	30.7	
12	3	15.8	4.0	225	1.6	8.0	3.180	20.1	28.5	
13	4	13.5	3.3	182	1.0	11.0	2.580	19.1	25.8	33.0
14	4	13.8	5.8	193	1.3	8.3	2.730	19.8		
15	5	13.3	2.3	230	3.9	3.5	2.640	19.9	31.6	30.5
16	5	13.0	2.4	250	4.0	3.2	2.290	17.6		
17	5	12.5	2.8	193	2.3	6.7	2.370	19.0	31.2	30.5
18	5	12.8	3.0	329	4.6	3.7	2.330	18.2	35.6	
19	5	12.5	3.8	303	.6	13.2	2.550	20.4	39.8	
20	5	12.5	4.3	340	.9	10.3	2.600	20.8	37.5	
21	5	11.5	4.5	280	.7	15.0	2.470	21.4	40.0	
22	6	10.8	3.0	314	1.5	7.7	2.350	21.8	33.7	33.7
23	6	10.1	3.1	303	1.9	5.0	1.910	18.9	30.2	
24	6	10.5	3.6	326	1.3	7.4	2.050	19.6	33.9	31.6
25	7	17.5	2.1	380	3.0	4.0	3.380	19.3	38.0	
26	8	14.5	3.0	208	.6	19.0	2.870	19.8	35.0	

the normal animal (table 1, dog 1) in which the inulin infusion was maintained for 12 hours with no appreciable increase in the inulin space supports this view.

Delay Time. The total inulin excreted after the infusion is discontinued constitutes not only the inulin in the extracellular space but also a certain amount contained within that portion of the urinary tract between Bowman's capsule and the bladder (dead space) at the instant of the cessation of the infusion. For the accurate measurement of the inulin space, the inulin in the dead space must be subtracted from the total recovery. This correction has been estimated in the following way.

During the infusion, control urine collections were made at intervals of 2 minutes. The infusion was then stopped at the end of one such interval, and similar col-

lections were continued for the next 10 or 15 minutes. If the inulin clearance is constant, the amount excreted per minute during the infusion is likewise constant.

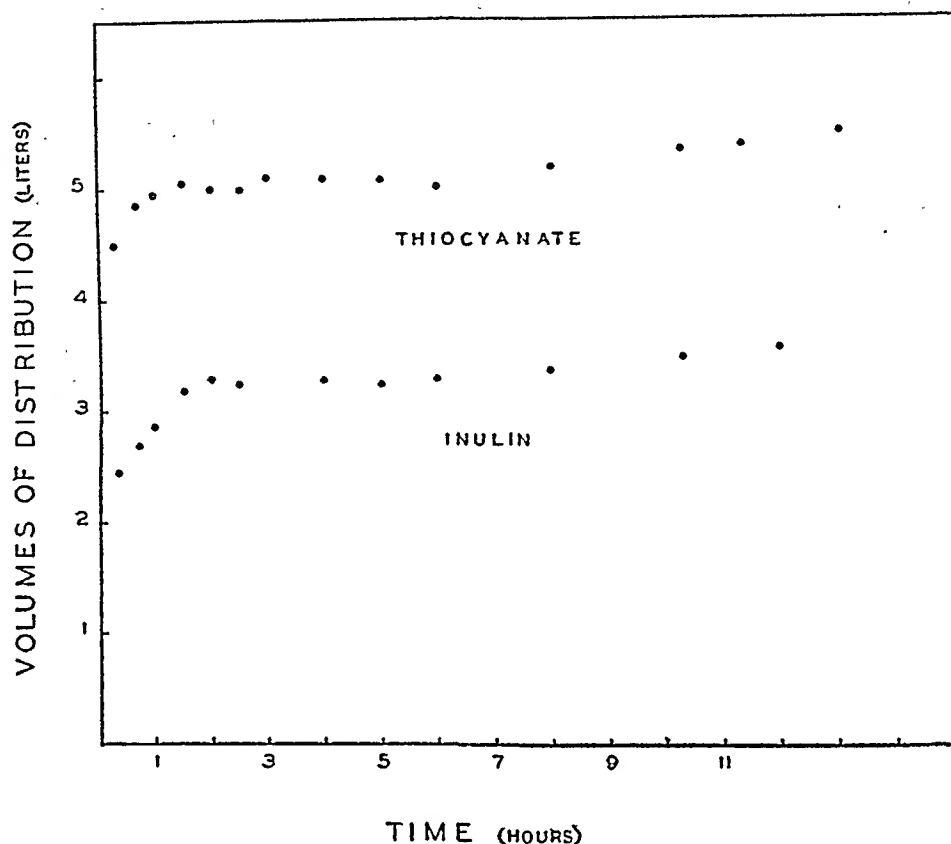


FIG. 1. EQUILIBRATION of inulin and thiocyanate in a nephrectomized dog. The injections were given at time zero. The dots represent calculated spaces of distribution from determined plasma levels. Two other dogs showed similar curves.

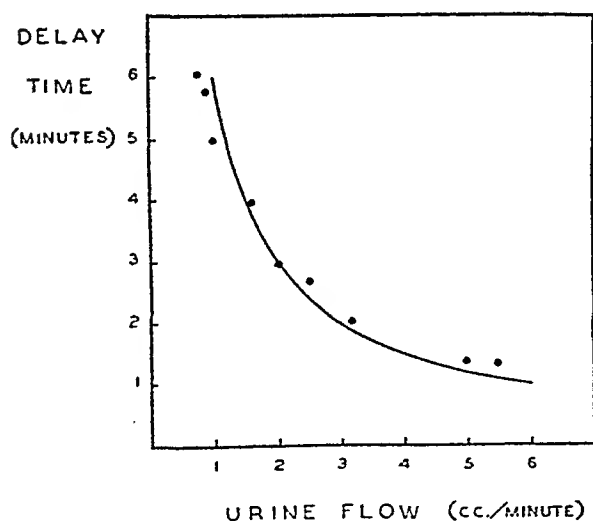


FIG. 2. RELATION between delay time and urine flow. The dots represent the results of 9 experiments performed in 4 normal dogs of similar size.

Experimentally it was determined that the plasma level falls immediately after the infusion is discontinued. The time elapsing between the fall in plasma level and the subsequent fall in the rate of excretion is the delay time. This time in minutes multi-

plied by the rate of excretion in mg. per minute equals the mg. of inulin in the dead space. The delay time was thus measured in 4 dogs with urine flows varying from 0.8 to 5.5 cc. per minute (fig. 2). It is clear that the delay time is inversely proportional to the urine flow, and that their product is therefore constant. This constant, equivalent to about 6 cc. in the dog, is a measure of the dead space.

The delay time is negligible when the urine flow is over 5 cc. per minute, it adds a 5 per cent error to the inulin space when the urine flow is 2.5 cc. per minute, and causes a larger error with urine flows less than 1 cc. per minute. All the inulin spaces reported in this paper have been corrected for appropriate delay times. The average correction in 26 experiments with varying urine flows amounts to 8 per cent of the uncorrected value (table 1).

Normal Values of the Inulin Space. The volume of distribution of inulin as determined by the above method was measured in 26 experiments performed in 8 normal dogs (table 1). In all instances, the infusion was maintained for at least 2 hours to insure complete and uniform distribution. The average corrected inulin space is 19.4 per cent of the body weight, with a range from 17.5 to 21.8 per cent.

TABLE 2. VOLUMES OF DISTRIBUTION OF INULIN, THIOCYANATE AND SODIUM²⁴ BEFORE AND AFTER TOTAL NEPHRECTOMY

CONTROL SPACES								SPACES AFTER NEPHRECTOMY							
Dog	Body wt.	Inulin		Sodium ²⁴		Thiocyanate		Body wt.	Inulin		Sodium ²⁴		Thiocyanate		
	kg	l.	% body wt.	l.	% body wt.	l.	% body wt.	kg.	l.	% body wt.	l.	% body wt.	l.	% body wt.	
A	15.0	3.900	26.0	4.650	31.0	5.640	37.6	13.3	3.460	26.0	4.590	34.6	5.700	43.0	
B	16.5	3.380	20.4			6.500	39.4	16.0	3.300	20.6	4.040	25.3	5.000	31.3	

The average thiocyanate space in these animals is 33.8 per cent of the body weight with a range of 25.8 to 40 per cent. The Na²⁴ space averages 30.4 per cent with a somewhat smaller scatter than seen in the thiocyanate values.

Nephrectomized Dogs. To corroborate the spaces obtained with the infusion method, control measurements of inulin, thiocyanate, and Na²⁴ spaces were made in normal anesthetized dogs, and then repeated after complete nephrectomy (table 2). The control inulin spaces were determined with the infusion method and the post-nephrectomy spaces with the single injection technique. The inulin space before and after nephrectomy proves to be the same (table 2).

DISCUSSION

The method here described assures the uniform distribution of inulin throughout the extracellular fluid, despite its rapid excretion.

The delay time correction has eliminated a source of error which was not considered in the preliminary paper (30). The average inulin space so corrected proves to be 19.4 per cent instead of the previously reported value of 21.6 per cent of the body weight.

The rate of excretion of the urinary inulinoid blank varies throughout the day.

This fact may introduce an error which can be avoided by elevating the plasma level 3- to 4-fold³, when the blank correction is reduced to negligible proportions.

The observations in control and nephrectomized animals confirm the inference that the infusion method measures the same space in the normal animal that the single injection method measures in the nephrectomized dog.

The arguments which favor the conclusion that inulin does not enter the cells have been mentioned above. Further, Wilde (13) has shown that in nephrectomized rats both inulin and sucrose, substances of vastly different molecular weights, have identical volumes of distribution.

Previous methods of measuring extracellular fluid have employed substances which are now known to enter cells or to be partially metabolized. The circumstance that the inulin space affords the lowest value of extracellular fluid yet recorded argues that it represents the best described approximation of that value. The only other alternative would be that inulin has diffused incompletely into the extracellular compartment. In table 1 it is demonstrated that if the infusion is maintained as long as 12 hours, a period approximately 6 times the required equilibration time, there is no further increase in inulin space. It is improbable that if after 2 hours diffusion is incomplete, it will remain so after 12 hours. In view of our results, we believe that inulin is distributed, at equilibrium, throughout the extracellular fluid volume.

Using deuterium oxide (33, 34), the total body water in 6 normal dogs has been found to average 63 per cent of the body weight, range 55.7 to 74.3 (35). As calculated by the difference between the total body water and the measurement of the extracellular fluid here reported, the intracellular water content averages 44 per cent, range 36.3 to 54.9.

From table 1 it is apparent that the ratio of the 3-hour Na^{24} space to the inulin space is approximately $3/2$. Consequently we conclude that about one third of the total sodium in the dog is intracellular.

SUMMARY

A new method has been devised which permits the use of inulin as a measure of the extracellular space, by means of an equilibrating infusion followed by the collection of the total inulin excreted, after the cessation of the infusion. The length of infusion necessary to insure uniform distribution of inulin throughout the extracellular space is 2 hours in the dog. A method is described to measure the delay time which permits the correction of the total inulin recovery. This delay time is inversely proportional to the urine flow. The product of delay time and urine flow is constant and a measure of the dead space. The dead space in the dogs used is 6 cc. The average extracellular space of the dog is 19.4 per cent of the body weight compared to 30.4 per cent and 33.8 per cent for the spaces of Na^{24} and thiocyanate respectively.

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³ A concentration of 30 to 50 mg. per cent does not exert any significant osmotic effect.

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RATE OF ENTRANCE OF URATE AND ALLANTOIN INTO THE CEREBROSPINAL FLUID OF THE DALMATIAN AND NON-DALMATIAN DOG¹

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OBSERVERS (1-3) have reported that the concentration of urate in cerebrospinal fluid (C.S.F.) is only a small fraction of that found in plasma or serum. The reason for this difference in concentration is unknown largely because of lack of precise information concerning (1) formation of the C.S.F. and (2) the physicochemical state of urate in the plasma. Although the weight of opinion (4-6) is that the blood-cerebrospinal fluid barrier possesses peculiarly selective properties, Wolfson *et al.* (3) recently postulated that the C.S.F. was essentially a dialysate or ultrafiltrate of plasma and that the small concentration of urate in it resulted primarily because of the relatively non-diffusible state of urate in plasma. This latter group, moreover, interpreted (7) the low rate of renal clearance of urate occurring in most mammals as resulting from inability of urate to pass the glomerular membrane, rather than from its partial renal tubular reabsorption.

Urate has been shown by us (8) to be diffusible through the glomeruli of the Dalmatian dog. In this animal, therefore, a direct determination may be made as to whether diffusibility of urate itself is of primary importance with regard to its entrance into the cerebrospinal fluid.

The concentration of allantoin in cerebrospinal fluid has not hitherto been determined. This substance is the principal excretory end product of purine metabolism in sub-primate mammals (except for the Dalmatian dog, where it shares this role with uric acid), and is therefore the physiological analog of uric acid in these animals. We have shown (9, 10) that allantoin is freely diffusible through the glomeruli of the rat, dog, and man. It was therefore of interest to us to determine the extent to which this freely diffusible substance is present in the C.S.F., and the rate at which it enters that fluid.

The results of our investigation indicate that some selective hindrance exists to the passage of both urate and allantoin from the plasma to cerebrospinal fluid of Dalmatian dogs, and a similar hindrance exists to the passage of allantoin and of injected urate from the plasma to the C.S.F. of non-Dalmatian dogs.

METHODS AND RESULTS

Concentration of Urate and Allantoin in the Plasma and C.S.F. of the Non-Dalmatian and Dalmatian Dog. Nineteen non-Dalmatian and 2 Dalmatian dogs were

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studied. Samples of C.S.F. (2 cc.) were obtained by the intraspinal insertion of a lumbar spinal needle (20 gauge) between the second and third cervical vertebrae of dogs anesthetized with sodium pentobarbital. Immediately after each sample of C.S.F. was obtained, a sample of blood was obtained, by puncture of the femoral artery.

Twenty-one pairs of samples of plasma and C.S.F. obtained from 18 non-Dalmatian dogs and 8 similar samples of plasma and C.S.F. from 2 thoroughbred Dalmatian dogs were analyzed for their urate content. Ten plasma and 11 C.S.F. samples obtained from 10 non-Dalmatian dogs and 5 similar samples of plasma and C.S.F. obtained from 2 Dalmatian dogs were analyzed for their allantoin content. Urate, allantoin and creatinine determinations were done according to previously described methods (8, 9).

The average plasma urate concentration of non-Dalmatian dogs was found to be 0.23 mg/100 cc. (S.E. mean \pm 0.016), with a range from 0.10 to 0.45 mg/100 cc. The average concentration of urate in the C.S.F. of these dogs was 0.24 mg/100 cc. (S.E. mean = \pm 0.014) with a range of 0.13 to 0.35 mg/100 cc. Although the average concentration and range of urate in the C.S.F. of normal dogs was approximately the same as that in their plasma, no correlation was established between the individual paired samples of plasma and C.S.F. Perhaps the necessary chemical error involved in the analyses of such minute samples led to this seeming lack of correlation.

The average plasma urate concentration of 2 Dalmatian dogs was 0.48 mg/100 cc. (S.E. mean = \pm 0.038) and ranged between 0.30 and 0.65 mg/100 cc. for the 8 samples. The average concentration of urate in the C.S.F. of Dalmatians was 0.24 mg/100 cc. (S.E. mean = \pm 0.027) and ranged from 0.18 to 0.30 mg/100 cc. The average concentration of urate in Dalmatian C.S.F. was identical with that in normal dogs, despite the fact that Dalmatian plasma urate concentration was twice that of normal dogs. The ratio of urate in plasma to urate in spinal fluid of Dalmatians varied from 1.6 to 3.0.

The average plasma allantoin concentration of non-Dalmatian dogs was 1.47 mg/100 cc. (range: 1.10 to 1.85 mg/100 cc.). The allantoin content of their C.S.F., however, was only 0.30 mg/100 cc. (range: 0.25 to 0.47 mg/100 cc.) or 80 per cent less than that of plasma. In the Dalmatian dogs, the average plasma allantoin concentration (0.68 mg/100 c.) was below that of the non-Dalmatian dogs but here also the average allantoin concentration of their C.S.F. (0.14 mg/100 cc.) was approximately 80 per cent below the plasma level.

In other words, there was a difference between the C.S.F. and plasma concentration of both urate and allantoin of normal Dalmatian dogs, and a similar difference in the allantoin levels of normal non-Dalmatian dogs; this difference is maintained despite the fact that plasma urate in the Dalmatian and plasma allantoin in all dogs are in diffusible form.

Rate of Entrance of Allantoin into the C.S.F. of Non-Dalmatian Dogs Following Elevation of the Plasma Allantoin Content. Although the above results indicated that under normal conditions allantoin was not distributed equally between plasma and C.S.F. of both types of dogs, it was thought desirable to study the change in concentration of allantoin in the C.S.F. after elevation of the plasma allantoin content.

Five non-Dalmatian dogs were anesthetized with sodium pentobarbital. Control blood and C.S.F. samples were taken, then a solution containing 500 mgm. of allantoin per 100 cc. of normal saline solution was infused intravenously at the rate of 3 cc. per minute. Blood samples were taken 30 minutes after the infusion had been started and again at the end of 90 minutes, at which time the second C.S.F. samples also were obtained. All samples were analyzed for their allantoin content.

As table 1 demonstrates, although the average plasma allantoin concentration in the 5 dogs during the perfusion period was 8.90 mg/100 cc., the average allantoin content of the C.S.F. at the end of the 90-minute period was only 0.68 mg/100 cc. as compared to the average initial concentration of 0.31 mg/100 cc. When compared to the gain in the creatinine concentration of C.S.F. resulting from a similar elevation of plasma creatinine in other dogs, it can be seen (compare tables 1 and 3) that approximately twice as much plasma creatinine had entered the C.S.F. in the same period of

TABLE 1. CHANGE IN ALLANTOIN CONTENT OF C.S.F. OF DOGS AFTER ELEVATION OF PLASMA ALLANTOIN BY INTRAVENOUS INFUSION

DOG	PLASMA CONC. OF ALLANTOIN BEFORE AND DURING INFUSION mgm./100 cc.				C.S.F. CONC. OF ALLANTOIN BEFORE AND DURING INFUSION mgm./100 cc.	
	Bef.	30 Min.	90 Min.	Av. ¹	Bef.	90 Min.
N1	1.5	14.2	17.1	10.9	0.31	0.69
N2	1.1	11.6	16.8	9.8	0.23	0.59
N3	1.5	10.9	13.5	8.6	0.25	0.75
N4	1.8	8.9	12.1	7.6	0.47	0.70
N5	1.5	11.1	10.0	7.5	0.30	0.69
Av.....	1.5	11.3	13.9	8.9	0.31	0.68

¹ Equals average concentration of allantoin in plasma during entire infusion (average of the preceding 3 determinations).

time. These observations suggested the presence of a selective barrier to the entrance of allantoin into the C.S.F.

This relative impermeability of the blood brain barrier to diffusible plasma allantoin was also shown in a second experiment in which 2 normal dogs were nephrectomized. As table 2 demonstrates, the average allantoin content of C.S.F. (3.81 mg/100 cc.) in the 2 dogs 72 hours after nephrectomy was still much less than that found in plasma only 24 hours after nephrectomy. Likewise the creatinine content of C.S.F. (2.11 mg/100 cc.) of the 2 dogs 72 hours after nephrectomy was less than half of that present in plasma only 24 hours after nephrectomy. However, the ratio of plasma creatinine to C.S.F. creatinine before and during the 72 hours following nephrectomy was always much less than the ratio of plasma allantoin to C.S.F. allantoin. These studies indicated that there was a failure to achieve equality between the concentration of allantoin in plasma and C.S.F. and a similar although less marked failure in the case of creatinine.

Rate of Entrance of Urate into the C.S.F. of Non-Dalmatian and Dalmatian Dogs Following the Elevation of Plasma Urate Content. Five non-Dalmatian dogs were anes-

thetized and after control blood and C.S.F. samples were obtained, they were given an intravenous infusion of a solution containing 500 mg. of urate, 200 mg. of creatinine, and 250 mg. of lithium carbonate per 100 cc. This infusion was given at the rate of 7 cc. per minute and maintained for 90 minutes. Blood samples were taken at 30 and again at 90 minutes, at which latter time a second C.S.F. sample was obtained. All samples were analyzed for their urate and creatinine contents. The same procedure was performed 4 times on 2 Dalmatian dogs.

Despite the lower average concentration of creatinine maintained during the period of infusion (table 3) approximately twice as much creatinine (0.67 mg/100 cc.)

TABLE 2. CHANGE IN ALLANTOIN AND CREATININE CONTENT OF PLASMA AND C.S.F. AFTER BILATERAL NEPHRECTOMY

TIME AFTER NEPHRECTOMY	DOG	PLASMA		C.S.F.		RATIO (PLASMA/C.S.F.)	
		ALLANT.	CREAT.	ALLANT.	CREAT.	ALLANT.	GREAT.
Hours		Mg/100 cc.		Mg/100 cc.			
Control	N6	1.57	0.58	0.26	0.52	6.05	1.13
	N7	1.94	0.76	0.44	0.50	4.44	1.52
Average.....		1.76	0.67	0.35	0.51	5.25	1.32 :
24	N6	11.8	3.90	0.98	1.10	12.1	3.55
	N7	14.2	4.70	0.99	1.11	9.0	4.25
Average.....		13.0	4.30	0.99	1.11	10.6	3.90
48	N6	18.7	6.79	2.17	1.49	9.0	4.60
	N7	23.0	7.29	2.96	1.62	7.4	4.60
Average.....		20.85	7.04	2.57	1.56	8.2	4.6
72	N6	25.3	9.20	3.54	2.23	7.2	4.1
	N7	27.6	9.30	4.07	1.98	6.8	4.7
Average.....		26.45	9.25	3.81	2.11	7.0	4.4

entered into the C.S.F. of non-Dalmatian dogs as did urate (0.39 mg/100 cc.). The same relative disproportion was observed (table 3) in the Dalmatian dogs. There seemed little question then that even the freely diffusible plasma urate of the Dalmatian dog was differentially hindered in its entrance into the C.S.F. Moreover, when the rate of urate transfer from plasma to C.S.F. of the non-Dalmatian dog was compared with that of Dalmatian, the two were observed (table 3) to be similar, suggesting the physicochemical similarity of the plasma urates in each type of dog.

Rate of Disappearance of Injected Urate from the C.S.F. of Non-Dalmatian Dogs. Five male dogs were anesthetized and immediately after blood and C.S.F. samples were obtained, 1 cc. of a solution containing 500 mg. of lithium urate and 500 mg. of creatinine per 100 cc. was injected into the cerebrospinal canal. Equal distribution of this solution throughout the C.S.F. was attempted by rapid withdrawal and re-

TABLE 3. CHANGE IN URATE AND CREATININE CONTENT OF C.S.F. OF DOGS AFTER ELEVATION OF PLASMA URATE AND CREATININE BY INTRAVENOUS INFUSION

DOG	AVERAGE PLASMA CONCENTRATION DURING INFUSION ¹		AVERAGE C.S.F. CONCENTRATION BEFORE AND AT END OF INFUSION				GAIN IN C.S.F. URATE AND CREA- TININE AT END OF INFUSION	
	Urate	Creat.	Bef. Inf.		End Inf. (2)		Urate	Creat.
			Urate	Creat.	Urate	Creat.		
Non-Dalmatian Dogs								
	Mg/100 cc.		Mg/100 cc.		Mg/100 cc.		Mg/100 cc.	
N8.....	10.9	10.1	0.25	0.60	0.83	1.42	0.58	0.82
N9.....	7.7	7.7	0.29	0.64	0.59	1.24	0.30	0.60
N10.....	6.8	7.7	0.27	0.64	0.76	1.31	0.49	0.67
N11.....	11.2	5.3	0.19	—	0.41	—	0.22	—
N12.....	11.8	4.6	0.24	0.69	0.62	1.26	0.38	0.57
Av.....	9.68	7.08	0.25	0.64	0.64	1.31	0.39	0.67
Dalmatian Dogs								
LD.....	8.4	8.1	0.18	0.49	0.62	1.55	0.44	1.06
LD.....	8.2	7.5	0.22	0.48	0.42	1.29	0.20	0.81
LD.....	12.0	5.8	0.30	0.58	0.77	1.13	0.47	0.55
SD.....	8.8	8.3	0.24	0.52	0.53	1.35	0.29	0.83
Av.....	9.4	7.4	0.24	0.52	0.59	1.33	0.35	0.81

¹ Equals average concentration of urate and creatinine in plasma during entire infusion (average of initial concentration, that after 30 minutes and that after 90 minutes of infusion).

² Equals concentration of urate and creatinine in C.S.F., 90 minutes after infusion had been begun.

TABLE 4. RATE OF DISAPPEARANCE OF INJECTED URATE AND CREATININE FROM C.S.F. OF DOGS

DOG	BEFORE INJECTION		AFTER INJECTION							
			30 Min.		60 Min.		120 Min.		24 Hours	
	Urate	Creat.	Urate	Creat.	Urate	Creat.	Urate	Creat.	Urate	Creat.
	Mg/100 cc.		Mg/100 cc.		Mg/100 cc.		Mg/100 cc.		Mg/100 cc.	
N13.....	0.20	0.49	53.4	55.2	37.1	40.6	22.9	23.9	—	—
N14.....	0.30	0.58	38.0	43.0	26.0	35.0	19.6	21.0	—	—
N15.....	0.20	0.44	19.6	21.0	13.0	16.0	7.4	8.0	—	—
N16.....	0.34	0.71	42.0	48.0	30.0	37.0	18.0	18.0	0.58	0.85
N17.....	0.28	0.60	31.0	37.0	19.0	19.0	11.0	9.2	0.38	0.73
Av.....	0.26	0.56	36.8	40.8	25.0	29.5	15.8	16.0	0.48	0.79

injection of 5 cc. of the C.S.F. 7 times by means of a syringe. Samples of C.S.F. were obtained 30, 60, and 120 minutes after this injection. Samples were obtained in 2 dogs 24 hours after injection. Blood samples also were obtained at 2 and at 24 hours

after injection and were analyzed for urate, allantoin, and creatinine. The samples of C.S.F. were analyzed for their urate and creatinine content.

Unlike the discrepancy observed between the rates of penetration of plasma urate and creatinine into the C.S.F., the rate of disappearance of these substances from the C.S.F. after injection therein was approximately equal (table 4). It is of interest that even after 24 hours not all of the excess urate or creatinine had disappeared from the C.S.F. However, the relatively small amount of either substance entering the blood from the C.S.F. could not be detected, as determinations of urate, allantoin, and creatinine in the plasma obtained 2 and 24 hours after injection from the dogs showed no essential change in the concentration of any of the 3 substances.

DISCUSSION

Recently Wolfson *et al.* (3) (assuming that C.S.F. was a modified dialysate or ultrafiltrate) conjectured that plasma urate was in a relatively non-diffusible state because of the relative paucity of urate in human C.S.F. On the basis of this latter conjecture, moreover, they were led to believe (7) that only a small fraction of total plasma urate was filtered through the glomerulus.

Our observations, however, were not in agreement with the above views. It appeared that the urate content of the C.S.F. was not strictly dependent upon dialysis or ultrafiltration of diffusible plasma urate but more perhaps upon the selective functions of the blood brain barrier. The content of urate in the C.S.F. of the ordinary dog and in the Dalmatian was the same although the plasma urate of the latter was twice that of the former and in a freely diffusible state (8). Moreover, when urate was given intravenously to these dogs, a similar degree of selective hindrance toward entrance of urate (as compared with the entrance of creatinine) into the C.S.F. was found in each type of animal, although the increased urate in the plasma of the Dalmatian was known to be in a diffusible state (8).

Similarly, despite the known diffusibility of allantoin in the plasma of both types of dogs (9), a marked discrepancy existed between the allantoin content of plasma and C.S.F. either at normal or at increased levels of plasma allantoin. This suggested again that the C.S.F. (in respect to allantoin also) was neither dialysate nor ultrafiltrate.

Our data therefore suggest that the low concentration of urate in C.S.F. of man is due not to the assumed non-diffusibility of plasma urate but to the demonstrated selective discrimination exercised by the blood brain barrier (4-6).

Moreover, considerable direct evidence is available that plasma urate in man and other animals is in a diffusible, hence filtrable state. Thus the concentration of urate in lymph (11), pleural fluid (12, 13), joint fluid (14, 15) and ascitic and pericardial fluid (12, 13) has been found to equal that of plasma. More importantly, Bordley and Richards (16) have demonstrated that the glomerular urine of the snake and frog contains as much urate as their plasma.

In view of these observations, it would seem hazardous to consider plasma urate either non-diffusible or non-filtrable through the glomerulus because of its low concentration in a fluid (C.S.F.) which has not been found to be either a true dialysate or ultrafiltrate (4-6).

SUMMARY

The allantoin and urate concentration in the cerebrospinal fluid of both non-Dalmatian and Dalmatian dogs was determined. The blood brain barrier of both types of dogs was found to be selectively discriminatory against the entrance of allantoin. However, the concentration of urate in the C.S.F. and plasma of non-Dalmatian dogs was the same at normal plasma levels, but when the plasma urate was artificially elevated, hindrance to the entrance of plasma urate into C.S.F. was observed and to the same extent as that found in the Dalmatian dogs. The state of diffusibility and glomerular filtrability of plasma urate in non-Dalmatian and Dalmatian dogs was discussed.

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REDUCTION OF PLASMA POTASSIUM CONCENTRATION OF THE DOG BY VIVODIALYSIS AND ITS RESTORATION IN NON-VISCERAL REGIONS

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IT HAS been found possible to remove sufficient potassium by vivodialysis within a few hours to indicate that potassium can move from other regions to replace that withdrawn from the extracellular fluid (1). Since the non-visceral regions, composed chiefly of skeletal muscle, skin, bone and associated connective tissue, contain the greater part of the body's potassium (2), it is of interest to determine whether or not they can supply potassium to sustain the concentration in the extra cellular fluid. To obtain evidence bearing on this question a study was made on the plasma potassium concentration in the arterial and femoral vein blood during the removal of potassium by vivodialysis.

The methods were essentially those reported previously (1). Small dogs were used in order to increase the relative capacity of the dialyzing equipment. This necessitated filling the dialyzer with heparinized blood from donor dogs before beginning the dialysis. It was also necessary to give small transfusions from time to time to compensate for sampling and incidental blood loss. These were given into the line carrying blood to the dialyzer so that the potassium was removed before it reached the dog. Blood for dialysis was drawn from the vena cava by a catheter passed through a femoral vein and returned through a cannula tied into a tributary of an external jugular vein. Arterial samples were taken from a 1 mm. O.D. polyethylene catheter (obtainable from Anchor Plastics Co., New York City) in the ipsilateral femoral artery and femoral vein samples from a similar catheter passed into the contralateral femoral vein in the distal direction. These catheters were passed into the respective vessels through needles introduced obliquely through the exposed vessel wall. When the needles were stripped off there was no serious bleeding. The catheters did not seem to interfere with blood flow. In two animals the intestinal mesentary was exposed by a short midline incision into the belly, and a slender polyethylene catheter was passed through one of the intestinal veins into the portal vein. The essential circumstances of each experiment such as the size of the animal and the extent of the dialysis are shown in table 1.

RESULTS AND DISCUSSION

More extensive removals of potassium relative to the size of the animal were achieved than in the earlier study (1) (table 1). These potassium removals effected substantial reductions in the arterial plasma potassium level (fig. 1). In some instances these ranged below the values reported in hypopotassemia due to potassium-

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deficient diets (3) or the treatment of diabetic coma with insulin (4). Only in patients suffering from periodic familial paralysis and given insulin or glucose have

TABLE I

EXPER.	WEIGHT	PUMP RATE		VOLUME OF DIALYZING FLUID USED	ELAPSED TIME OF DIALYSIS	EXTRA BLOOD GIVEN		TOTAL K IN DIALYZING FLUID	K REMOVED FROM DOG	K IN EXTRACELLULAR FLUID INITIALLY
		Blood	Dialyzing fluid			Volume	Plasma K content			
	kg.	cc/min.		l.	min.	cc.	mg.	mg.	mg.	mg.
I ♂	10.9	93-98	239	51.9	219	350	30	723	693	513
II ♀	7.2									
III ♂	8.8	97-103	267	69.1	262	500	53	1099	1046	470
IV ♀	10.3	86-97	239	88.5	461	400	41	1523	1482	515
V ♀	10.0	54-102	232	94.0	429	880	110	1656	1546	429
VI ♀	10.0	90-101	219	87.5	399	600	75	1482	1407	459

Elapsed time of dialysis includes the time during which dialysis was interrupted. The potassium in extracellular fluid initially was calculated by multiplying the initial concentration in the plasma by 30% of the animal's weight. The potassium removed from the dog equals the total amount in the dialyzing fluid minus that in the plasma of the extra blood given. All of the animals were allowed to fast for about 2 days prior to dialysis.

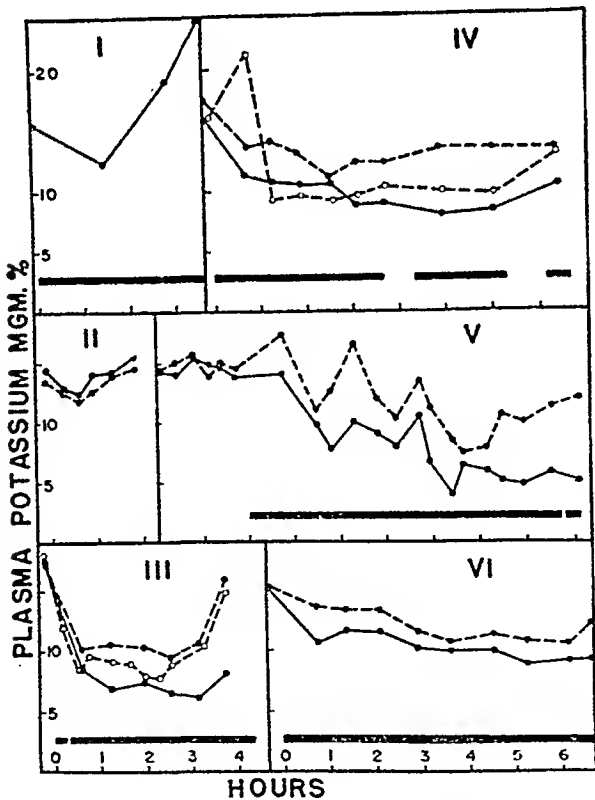


Fig. 1. DOGS WERE UNDER SODIUM PENTOBARBITAL ANESTHESIA and heparinized in all instances. In *experiment I*, the blood was both drawn and returned to the posterior vena cava. Although the return was proximal, yet the pump rate for blood was sufficiently great to cause retrograde flow in the vena cava and sufficient recirculation to reduce the efficiency of dialysis in the smaller size of dog. The last samples taken in all experiments were agonal. Arterial: solid lines, solid circles; femoral vein: broken lines, solid circles; portal vein: broken lines, open circles. Horizontal bars indicate the periods during which the dialyzer was operating.

equally low values been found (5). The evaluation of this finding, however, would necessitate a comparative study of the methods used in determining potassium. The predialysis values, however, were within the range found by others (6) for dogs under sodium pentobarbital anesthesia.

The femoral vein plasma potassium was sustained at a level distinctly higher than the arterial during the period when this was reduced by potassium removal. The difference was so large and maintained over such prolonged periods that blood flows so small as to be unlikely must be assumed in order to explain it on any basis other than the movement of potassium into the extracellular fluid within the region. Skeletal muscle, skin, bone and erythrocytes may be considered as possible sources of this potassium. Since both the total amount and the concentration of potassium in the erythrocytes in the body of the dog are small (7), they seem to be unlikely as a source. Conversely because of both the high concentration and the large total amount within skeletal muscle, this tissue must be considered first among likely sources of potassium (2). The result reported here is in accord with earlier studies in which it was found that the hind limbs of the frog would give up potassium to a perfusing fluid poor in potassium (8).

Several factors have been found to affect the plasma potassium concentration (9). Some one or combination of these probably was responsible for the fluctuations appearing in the femoral vein concentrations. An agonal rise, probably on the same basis, was apparent in most of the experiments.

The smaller arterio-venous increases found in the portal vein samples may indicate relatively as great a mobilization of potassium from the viscera drained as from the hind limb if the difference in the rate of blood flow is taken into account (10).

SUMMARY

The arterial plasma potassium level of the dog was substantially reduced by vivodialysis. Concurrently there was a definite arterio-venous increase in the plasma potassium concentration of blood flowing through the hind limb.

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CREATINURIA FROM GUANIDOACETIC ACID INGESTION AND ITS RELATION TO THE SITE OF ACTION OF METHYLTESTOSTERONE¹

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IN NO experiments in which creatine has been administered to man or other animals has the administered creatine been recovered quantitatively either as creatine or creatinine (1, 2). Three explanations for the apparent loss of administered creatine have been offered: 1) that it may be converted to some compound other than creatine, 2) that it may be partly destroyed in the intestine by bacteria, and 3) that it may retard the synthesis of creatine in the body. The first hypothesis is discredited by the experiments of Bloch and Schoenheimer (3) and du Vigneaud (4) with isotopic creatine. The second hypothesis, emphasized by Bodansky (5), has been tested only under limited conditions. Recently in this laboratory a subject, whose tissue-creatine had been labeled with N¹⁵, ingested large quantities of non-isotopic creatine (6). The ingested creatine could not be accounted for in either the tissue depots or the excreta. It appeared that during and for approximately 10 days after the ingestion of creatine the synthesis of endogenous creatine was greatly retarded.

In keeping with this inference the excretion of guanidoacetic acid, the precursor of creatine, increased. To account for the total deficiency in the production of creatine it was suggested that guanidoacetic acid is not entirely converted to creatine. Studies of the effect of methyltestosterone, however, indicated that this compound accelerates the synthesis of guanidoacetic acid, which in turn is methylated to creatine (7, 8). Creatinuria appears when the capacity of the tissues to store creatine is exceeded. The results of the two sets of experiments seemed to be contradictory. If the presence of an excess of creatine inhibits the methylation of guanidoacetic acid, the synthesis of endogenous creatine should be reduced to minimum when the tissue depots are saturated with creatine. To resolve the conflict it is necessary to postulate either that methyltestosterone accelerates the methylation as well as the synthesis of guanidoacetic acid or that there is an alternate route for the disposition of excess creatine in the body.

In the present experiments tolerance to guanidoacetic acid was studied before and after ingestion of creatine to determine whether its methylation was in fact retarded by excess creatine. The conversion of guanidoacetic acid to creatine was also studied after the tissues had been loaded to capacity with creatine. Under these conditions there was no significant evidence that the conversion of guanidoacetic acid

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to creatine was retarded. In fact, it has been possible to simulate the creatinuria of methyltestosterone simply by administering guanidoacetic acid. In addition it has been found that in man an increase in reabsorption of creatine by the tubules of the kidneys is associated with a reduction in reabsorption of guanidoacetic acid (9). This provides an alternate explanation for the increased excretion of guanidoacetic acid during administration of creatine.

PROCEDURE

The author served as subject and, except during periods in which large quantities of creatine were ingested, received a diet free from creatine, which contained an average of 90 gm. of protein and calories adequate to maintain weight. This was supplemented with 250 cc. of milk per day. To simulate physiological conditions as closely as possible when creatine or guanidoacetic acid (GAA) was ingested over the periods indicated in the figures, the quantities were divided into 16 portions which were taken in capsules at hourly intervals. In the case of daily quantities greater than 2.5 gm. 32 portions were taken at half-hour intervals over the same period. Twenty-four-hour urine collections were terminated with the over-night specimen approximately at the same hour each day, and all values were corrected to the quantity excreted per 24 hours. When GAA tolerance tests were made, 1.5 gm. were ingested in 5 equal hourly doses at the beginning of the collection period. Single blocks in the figures extending over several days represent instances in which daily urine collections were pooled by taking aliquots proportional to their volumes in order to prevent summation of analytical errors. When single daily urines were analyzed there was considerable fluctuation in the values for creatine. Since each low value was followed by a correspondingly high value and the averages showed a high degree of consistency, it is assumed that the fluctuations were due to incomplete voiding of the concentrated overnight urine.

METHODS

The analytical methods for GAA and for creatine and creatinine have been described in detail in a previous paper (9). All values given for creatine were corrected for GAA present in the samples and vice versa. To minimize spontaneous conversion of creatine to creatinine urine specimens were kept at 4° after collection and were analyzed with minimal delay. During the period in which 10 gm. of creatine were ingested daily, the urines were kept frozen until analyzed. Thymol was used as preservative, and analyses were done before bacterial growth was evident. All urines were extracted with chloroform after dilution to remove traces of protein before analysis for GAA. The GAA was synthesized by the method of Nencki and Sieber (10) and gave theoretical values for nitrogen on analysis.

CALCULATIONS

In previous studies on the same subject over a period of several months the fraction of the body creatine excreted as creatinine per day was found to be 0.0164 (6). Therefore, in estimating changes of body creatine, the average daily creatinine excretion, expressed as creatine, during the last 4 days of each period has been divided by

this factor. The average basal excretion of GAA for this subject was 100 mg. per day. In calculating the recovery of ingested GAA, this quantity has been subtracted from the daily excretion to give the excess GAA excreted. Creatine or GAA excreted as excess creatinine has been calculated by subtracting the average basal creatinine excretion of the subject, 1.96 gm. from the total daily excretion, all values for creatinine being expressed as the creatine equivalent. When heavy creatinuria was present, spontaneous conversion of creatine to creatinine during the interval between collection of urine and analysis may make this value somewhat higher than the actual value, but this increment is exactly balanced in the analytical procedure by the concomitant reduction in the values for creatine.

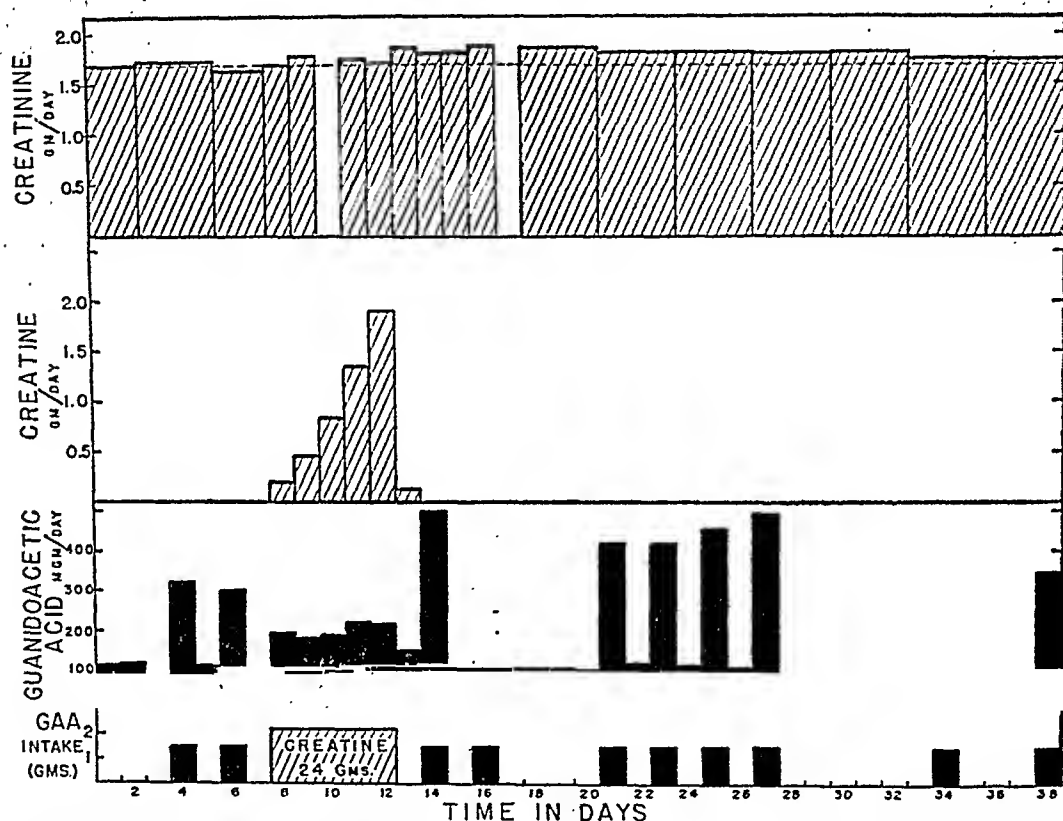


FIG. 1. TOLERANCE TO GUANIDOACETIC ACID before and after ingestion of creatine. The initial level of creatinine excretion is indicated by the dotted line.

RESULTS

In figure 1 are shown the results of a series of GAA tolerance tests made before and after the ingestion of 24 gm. of creatine over a 5-day period. This experiment was designed to duplicate the previous study made in this laboratory in which a similar amount of creatine was given to the same subject after the tissue creatine had been labeled with N-15. A slightly smaller quantity, 19.4 as opposed to 22.6 gm., of creatine was retained than in the previous experiment. This may be explained by the fact that the subject had been on a diet free of creatine for a shorter period prior to the experiment. Since the previous studies had suggested a retardation of creatine synthesis during and following the ingestion of the non-isotopic creatine, it was ex-

pected that GAA given shortly after the creatine ingestion would appear in larger amount in the urine. Such was in fact the case; twice as much GAA in excess of the basal excretion appeared in the urine when tolerance tests were made after the creatine ingestion as before. But the results of subsequent tolerance tests were inconsistent in several respects with the concept that creatine synthesis was retarded. Successive tests made on the 9th to the 15th days following the creatine ingestion showed successively decreasing instead of increasing tolerance during the time when it would be expected from previous data that endogenous synthesis would have returned to initial values. In addition, the creatinine excretion remained elevated for as long as GAA continued to be ingested. From an initial value of 1.69 gm. the daily creatinine output was elevated to an average of 1.82 gm. during the period immediately following the creatine ingestion. From the 12th to the 20th day following the creatine ingestion this averaged 1.82 gm. per day. Previous data had shown that creatinine excretion and the tissue creatine from which it is derived would normally have returned almost to the initial value by the end of this latter period. Thus it is inferred that a large portion of the 9 gm. of GAA given in the tolerance tests subsequent to the creatine ingestion was methylated to form creatine.

Before testing this inference further, the subject's tissues were first saturated with creatine by the ingestion of 90 gm. over a period of 9 days. Of this quantity 33 gm. were retained. Creatine ingestion was then continued at the rate of 1 gm. per day. Even this small quantity taken in 60-mg. portions at hourly intervals was found sufficient to sustain a minimal but definite creatinuria. Serum creatine likewise was elevated to a value of 0.85 mg. per cent at the end of the 57th day, 8 hours after the last 60 mg. dose of creatine. Thus a portion of any creatine formed from exogenous GAA would be expected to appear in the urine. After the creatinuria had approached a basal quantity, 1.5 gm. of GAA were added to the 1.0 gm. of creatine on the 52nd, 54th and 58th days. As is shown in figure 2 on each occasion the excretion of creatine was significantly increased. On the 63rd day 2.5 gm. of GAA alone were substituted for the creatine ingested. Creatinuria persisted and gradually increased to over 0.5 gm. by the 8th day after starting the ingestion of GAA alone and averaged 0.62 gm. for the latter half of the period. The creatinine excretion did not fall during this time, which suggests that the creatine of the urine was not derived from the tissue depots. The average daily creatinine excretion from the 58th to the 62nd day, when creatine alone was ingested was 1.89 gm.; that during the last 13 days of GAA ingestion was 1.96 gm. Since the creatinuria was greater during this latter period, it is possible that a part of this apparent increase may represent spontaneous conversion of creatine to creatinine. In the presence of over 10 times as much urinary creatine on days 39 through 47, however, creatinine values did not exceed 1.97 gm. daily.

The constant ingestion of GAA with production of creatinuria withdraws methyl groups from the body. To determine whether this depletion of labile methyl was a factor limiting the quantity of creatine formed from the GAA, the dietary protein was restricted to 60 gm. per day during the first 11 days of GAA ingestion. Six gm. of DL-methionine were added to the diet during the last 2 days to provide a source of methyl groups. The creatinuria did not change during this latter period, the 72nd and 73rd days shown in figure 2.

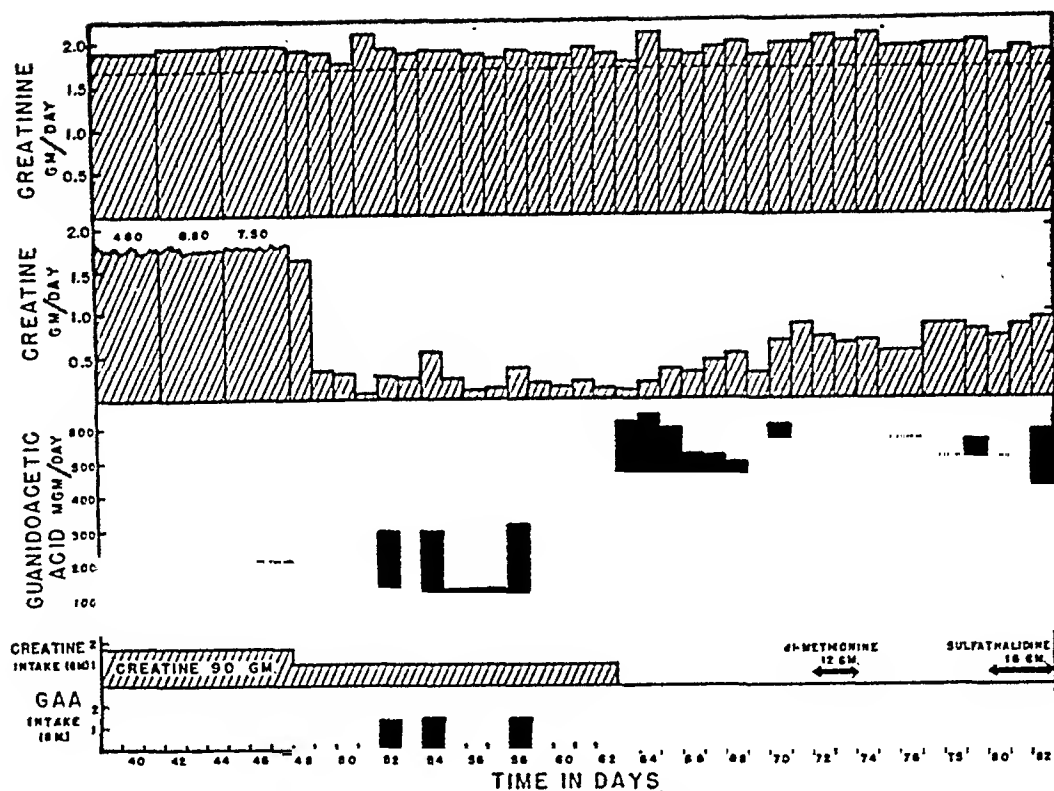


FIG. 2. PRODUCTION OF CREATINURIA from ingestion of guanidoacetic acid. The initial level of creatinine excretion is indicated by the dotted line. On the morning of the 58th day serum creatine was 0.85 mg.% and serum creatinine 1.4 mg.%.

TABLE 1. RECOVERY OF INGESTED CREATINE AND GUANIDOACETIC ACID

	PERIOD			
	I	II	III	Total
	Days 1-38	Days 39-62	Days 63-82	Days 1-82
	gm.	gm.	gm.	gm.
Creatine ingested.....	24.0	104.9	0	128.9
Guanidoacetic acid ingested.....	16.8	4.9	56.0	77.7
Total intake.....	40.8	109.8	56.0	206.6
Creatine excreted.....	4.8	61.5	9.9	76.2
Guanidoacetic acid excreted in excess of basal excretion of 100 mg/day.....	4.4	2.4	10.2	17.0
Creatine excreted in excess of basal excretion of 1.69 gm/day.....	4.3	6.3	6.0	16.6
Estimated increase in body creatine based on creatinine excretion (see text).....	5.0	9.5	1.0	15.5
Total creatine and/or guanidoacetic acid accounted for:				
Total.....	18.5	79.7	27.1	125.3
Per cent.....	45	73	48	61
Grams per day of ingested creatine or guanidoacetic acid not accounted for.....	0.59	1.26	1.44	0.99

All values for creatinine and guanidoacetic acid are expressed as the creatine equivalent.

Since Bodansky (5) and Twort and Mellanby (11) had isolated from intestinal contents Gram-positive bacteria capable of rapidly destroying creatine, the subject took sulfathalidine for a 3-day period in an attempt, during GAA ingestion, to augment the creatinuria. This did not change, as can be seen during days 79 to 82 of the same figure.

In table 1 is given the over-all balance between ingested and excreted creatine and GAA, together with an estimation of changes in body creatine. The method of calculation is described above. Of the total intake 39 per cent cannot be accounted for or recovered. The study has been divided into three periods. In *Period I* (days 1-38, corresponding to fig. 1) during which 24 gm. of creatine were ingested and GAA tolerance tests were done, 45 per cent of the ingested compounds could not be accounted for. In *Period II* (days 39-62 of fig. 2) 105 gm. of creatine and 5 gm. of GAA were ingested. The greater part of the large quantity ingested during the first 9 days was excreted directly as creatine so that the percentage accounted for was high (73%). But of the 43.4 gm. of creatine actually retained only 31 per cent was accounted for. In *Period III* (days 63-82 of fig. 2), only GAA was ingested. Of the 45.8 gm. of GAA, expressed as creatine, which was not excreted directly as GAA, 9.9 gm. were excreted as creatine; there was an apparent increase in the tissue creatine of 1.0 gm., and 6 gm. of extra creatinine were excreted. Thirty-seven per cent of the retained GAA was accounted for. The fact that in the preceding period the recovery of creatine itself was incomplete suggests that the actual quantity of GAA converted to creatine may have been greater.

DISCUSSION

During the latter two periods of the above experiment the body creatine was at or close to the maximum value for the subject. An average of 1.26 gm. per day of creatine could not be accounted for during the second period, when creatine was ingested uninterruptedly. To account for this discrepancy on the basis of an inhibition of GAA methylation to creatine, there would have to be roughly a 65 per cent inhibition of the methylation of the 1.9 gm. of endogenous GAA synthesized per day. However, in the present study a creatinuria of from 0.5 to 0.9 gm. per day was produced by the ingestion of GAA under these conditions, and 37 per cent of the GAA not excreted as such could be accounted for as creatine or creatinine. When creatine itself was ingested in the preceding period, 31 per cent of the comparable amount which was retained could be accounted for. The calculations are certainly only approximations, but they suggest that there was no essential difference in the manner in which the two substances were metabolized. If GAA were diverted under these conditions to a metabolic pathway other than the formation of creatine, one would expect on comparing a period of creatine with one of GAA ingestion to recover much less of the GAA than of the creatine. The findings are consistent with a conversion of a major portion of the retained GAA to creatine.

The rise in GAA excretion which occurs when creatine is ingested can be explained on the basis of a competition by the two substances for renal tubular reabsorption (9). The creatinuria from GAA ingestion, however, cannot be explained on this

basis for two reasons. Administration of GAA during clearance studies did not reduce the reabsorption of creatine by the renal tubules (9). Moreover, under the conditions of the present experiment creatinine excretion increased or remained constant during the periods of GAA ingestion, suggesting that the body creatine was not decreased.

The metabolic fate of that portion of administered GAA or creatine which has not been accounted for by balance or isotope studies remains obscure. The answer may lie in the possibility emphasized by Bodansky (5) that a portion of the creatine transported to or formed in the liver is secreted with the bile and is thus subject to bacterial destruction in the intestines before reabsorption. In his experiments on rats he showed that after a single 100-mg. dose of GAA the creatine of the intestinal contents determined by the enzymatic method was increased five times above normal values at 12 hours and remained double the normal value at 24 hours. It seems possible that the small amounts of N-15 recovered in the urinary ammonia after feeding labeled creatine to rats (3) and to man (6) is derived from creatine destroyed in the gut. There is evidence suggesting that the liver serves as a primary reservoir for exogenous creatine before its more gradual transfer to muscle (6). With greater than physiological quantities, a larger proportion may thus be subject to excretion in the bile and subsequent bacterial destruction. That a larger quantity of N-15 is recovered in the urinary ammonia and urea on administering comparable quantities of GAA to man (6) might be explained by the fact that GAA must first be converted to creatine in the liver, whereas exogenous creatine may directly enter muscle. Since the organisms capable of destroying creatine which were described by Twort and Melanby (11) and by Bodansky (5) are anaerobes and possibly not inhibited by sulfonamides, the negative results with sulfathalidine in the present experiment is not conclusive.

During these experiments the maximum creatinine excretion averaged over any 5-day period following creatine ingestion was 2.00 gm. per day. Assuming that the subject excreted the same proportion of his body creatine as during the previous isotope experiments, or 1.64 per cent, this would correspond to a total body creatine of approximately 140 gm. This represents a 20 per cent expansion of the initial body creatine and appeared to be the maximum for the subject.

In 1921 Gibson and Martin (12) noted an increase in apparent creatinuria during one day in which GAA was fed to a child with muscular dystrophy. Similarly, in this laboratory we have produced creatinuria by feeding GAA to a patient with myotonia atrophica who had shown inability to retain administered creatine (13). In the present study creatinuria has been produced by administration of GAA to a normal subject. This is compatible with the concept that methyltestosterone produces its effect on creatine metabolism solely by accelerating the synthesis of guanidoacetic acid.

SUMMARY

In experiments during which creatine and guanidoacetic acid were ingested by a normal subject no evidence was obtained suggesting that methylation of guanidoacetic acid to form creatine is retarded when an excess of creatine is present. It was

possible to produce a substantial creatinuria by the administration of guanidoacetic acid alone, thus simulating the effect of methyltestosterone on creatine metabolism. This suggests that the sole action of methyltestosterone on creatine metabolism is in acceleration of guanidoacetic acid synthesis.

The author is indebted to Dr. J. P. Peters and to Dr. H. D. Hoberman for valuable advice and criticism.

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EFFECT OF MAMMALIAN (POSTERIOR LOBE) PITUITARY EXTRACT ON WATER BALANCE OF FROGS WHEN PLACED IN DIFFERENT OSMOTIC ENVIRONMENTS¹

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THE frog lives in a hypotonic environment, while maintaining body fluids of considerable osmotic activity. There is an osmotic gradient between the external water and the internal body fluids, which tends to force water into the tissues. At the same time, the frog skin is an important respiratory membrane which must be permeable to gases. This also makes impermeability to water improbable. The frog skin is in fact somewhat permeable to water, which continually flows into the frog's tissues (1).

The continual dilution of the internal environment is amended by excretion of a large volume of very dilute urine (2). Osmoregulation is attained by a balance between these two processes. Under normal conditions the skin offers a resistance to the inward passage of water (3), which spares the necessary osmotic work otherwise imposed on the kidney.

Several factors are known to disturb the water balance of frogs living in water. Low temperature (4), anaesthesia (5, 6), destruction of part or all of the central nervous system (7), and flaying (5) all cause an increase in body water.

Large doses of mammalian posterior lobe pituitary extract cause summer frogs to gain about 20 per cent in weight in 3 hours. The weight then declines to normal or lower in about 12 hours (8). The hormone responsible is probably different from either the vasopressor or oxytocic principles, although associated with the latter. The hormone is more abundant in the lower vertebrates than in mammals, and has been described in extracts of crustacean eye-stalks (9, 10).

Pituitary extract increases the rate of weight gain of frogs in which the escape of urine is prevented by anal ligatures (11). Therefore the effect is due (at least partially) to an increased rate of influx of water compared with normal. The extract seems to lower the resistance of the skin to the inward diffusion of water. Reports also exist which indicate that pituitary extract inhibits water loss in the frog (12).

This investigation concerns the effect of posterior lobe pituitary extract on the water balance of frogs when placed in different osmotic environments.

METHODS

Frogs (*R. pipiens*) were taken at random from storage tanks. They were weighed roughly to the nearest 0.1 gm. and arranged in order of increasing weight. Alternate frogs were then assigned to experimental and control groups. This bal-

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anced the body weights between the groups, and eliminated a source of variation. There were usually 10 frogs in each group.

In some experiments purse string sutures were tied through the perianal skin. This usually prevented the escape of urine. Occasionally a leak developed, and the frog was discarded from the experiment. By this procedure the urine was weighed with the frog, and the results obtained then referred only to the rate of exchange of water through the skin.

A commercial mammalian posterior lobe extract was used throughout (Pituitrin S., Parke, Davis). This extract contains both vasopressor and oxytocic principles. The dosage was chosen to give a maximum increase in body weight, according to the dosage-response curve of Boyd and Young (13). This was 1.0 I.U. per 10 gm. of body weight, by injection in the dorsal lymph sac.

The osmotic environment was varied by placing the frogs in sucrose solutions of three different concentrations. Sucrose was used for two reasons: 1) since frog skin actively transports a number of ionized substances, a non-electrolyte was desirable; 2) the frog skin is probably relatively impermeable to sucrose.

Three levels of osmotic activity were used: hypotonic, isotonic and hypertonic. The hypotonic solution used was simply distilled water. In this case the osmotic gradient forces water inward, and is equal to the osmotic activity of the frog tissues. The osmotic activity of the internal environment of the frog is about 0.43°C . (14) corresponding to a pressure gradient of 5.2 atmospheres.

Isotonic sucrose was taken to be 0.23 molar. In this solution, the osmotic gradient across the frog's surface is approximately zero.

The hypertonic solution of sucrose was made up to 0.46 molar, just twice the isotonic concentration. This approximately reverses the normal osmotic gradient, and forces water outward from the tissues into the solution. The frogs were kept in 100 cc. of the appropriate solution, in individual jars with perforated tops. The size of the jars was such that a frog in a sitting position could keep the external nares at the surface of the solution.

Weighings were performed at suitable intervals after the beginning of the experiment. For weighing, a frog was removed from the solution, and dried lightly on a towel. The urine was expressed in the case of a normal frog. If an anal ligature had been placed, it was inspected for leaks. The frog was then weighed to the nearest 0.1 gm. on a beam balance, and replaced in the solution.

RESULTS

The weight changes observed were expressed as percentages of the original weight of the frog at the beginning of the experiment. The mean percentage weight increases are plotted against time in figures 1 to 6.

Figure 1 shows the weight-time curves for frogs in distilled water, with and without the injection of pituitary extract. The extract caused a typical transient weight gain, followed by a return toward normal. The controls lost weight slightly, probably related to the handling of the frogs.

Figure 2 shows the result of the same experiment, with anus-ligated frogs. The controls (ligature only) gained weight at the rate of 1.8 per cent of body weight per

hour. This represents the normal rate of influx of water into the frogs and the normally equal rate of excretion of urine. Pituitary extract increased this normal rate of

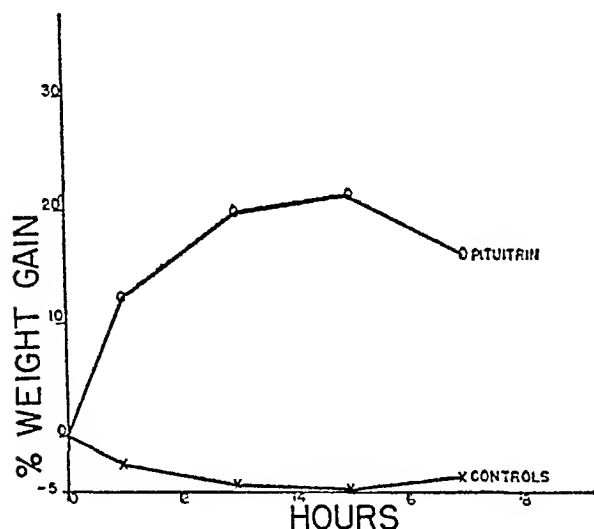


Fig. 1. MEAN WEIGHT CURVES FOR NORMAL FROGS in water, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

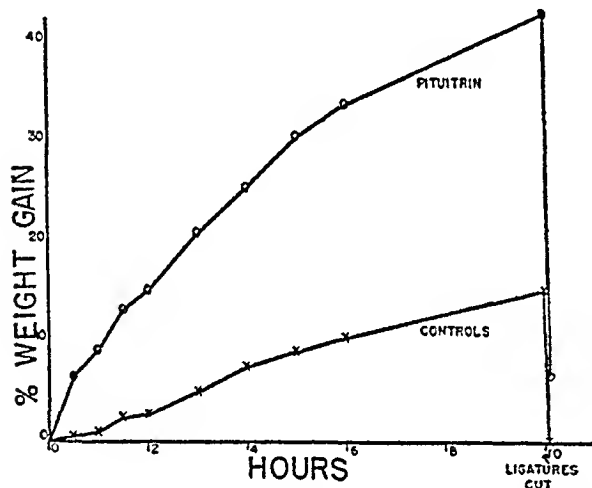


Fig. 2. MEAN WEIGHT CURVES FOR FROGS WITH ANAL LIGATURES, in water, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm body weight.

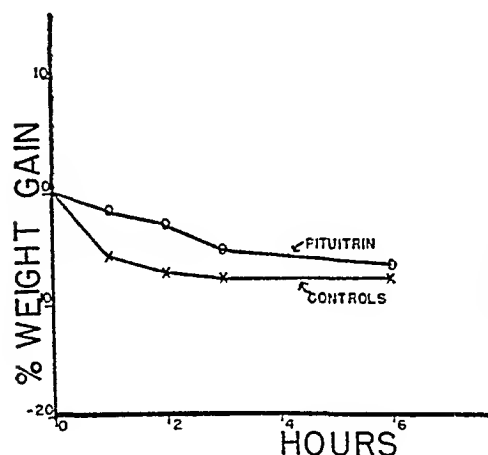


Fig. 3. MEAN WEIGHT CURVES FOR NORMAL FROGS in isotonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

influx to about 7.5 per cent of body weight per hour. When the ligatures were cut, there was a prompt and marked loss of urine.

Figure 3 shows the weight-time curve for frogs in 0.23 molar sucrose solution

(isotonic), with and without pituitary extract. In the controls there was a rapid loss of weight in the first 2 hours, after which the weight became constant. The pituitary

Fig. 4. MEAN WEIGHT CURVES FOR FROGS WITH ANAL LIGATURES, in isotonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

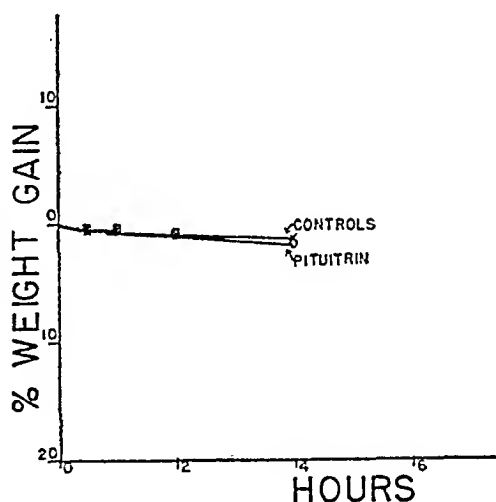


Fig. 5. MEAN WEIGHT CURVES FOR NORMAL FROGS in hypertonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

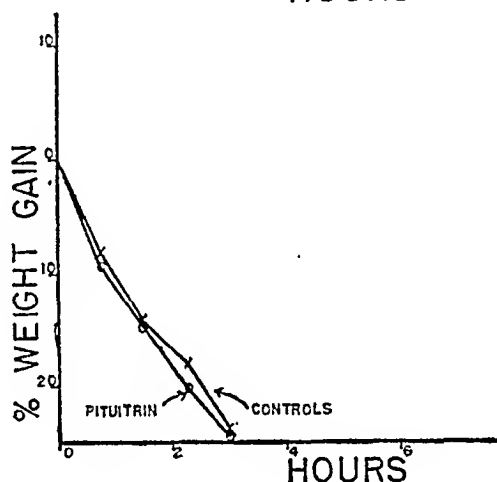
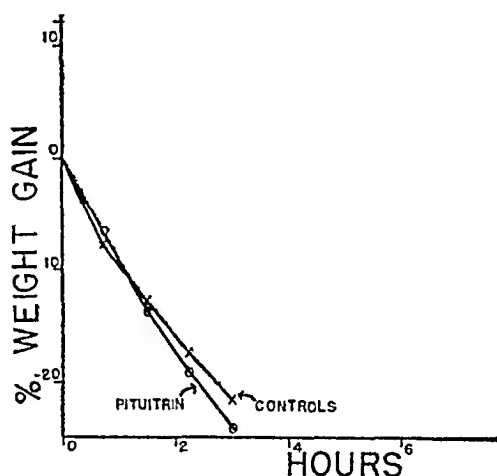


Fig. 6. MEAN WEIGHT CURVES FOR FROGS WITH ANAL LIGATURES, in hypertonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.



extract retarded this weight loss, although the final result was the same within experimental error. Figure 4 shows the result of the same experiment with anus-ligated frogs. In this case there was no appreciable weight loss, and no change due to pituitary extract.

Figure 5 shows the result when the normal osmotic gradient was reversed by placing the frogs in 0.46 molar sucrose solution. The weight loss was rapid, about 8.5 per cent of body weight per hour. Injection of pituitary extract caused no significant difference in the rate of weight loss. Figure 6 shows the result of the same experiment in anus-ligated frogs. The curves are practically identical with those of figure 5, i.e. neither anal ligature nor pituitary extract changed the rate of weight loss of frogs in 0.46 molar sucrose solution.

DISCUSSION

Consider first the water exchanges of frogs when subjected to the three levels of ambient osmotic activity: *a*) hypotonic, *b*) isotonic and *c*) hypertonic.

Hypotonic. For a normal frog in water, a steady state is attained when the influx of water through the skin is balanced by the excretion of urine. If the anus is blocked by a ligature, the weight increase over the next few hours is a measure of the normal influx and loss of water through the frog. From figure 2, this is found to be approximately 1.8 per cent of body weight per hour. This rate depends on 2 factors: 1) on the magnitude and direction of the osmotic gradient between the external solution and the tissues, and 2) on the resistance of the intervening tissues to the flow of water.

Isotonic. When frogs are transferred from water to isotonic sucrose solution, they lose weight for about 2 hours and then establish a new steady state (fig. 3). If the escape of urine is prevented, this adjustment does not take place (3). In the absence of an osmotic gradient the influx of water ceases, as is obvious from figure 4, but the excretion of urine continues for a short time, causing some weight loss as shown in figure 3.

Hypertonic. When frogs are transferred from water to 0.46 molar sucrose solution, the normal osmotic gradient is approximately reversed. There results a rapid weight loss of about 8.5 per cent of body weight per hour, which is not modified by anal ligature. This loss must therefore take place through the skin, assuming no loss through gastro-intestinal tract. The rate of outflow of water under these conditions is about 4.7 times the inflow when the gradient is in the normal direction, as shown by comparing figures 2 and 6. Since the osmotic gradients are approximately equal and opposite, the difference must lie in the resistance of the tissues to the flow of water in opposite directions. The greater resistance to inflow is of obvious advantage to the frog since it reduces the amount of urine which would otherwise have to be excreted. The exact nature of the resistance is unknown, although speculative hypotheses have been proposed (1).

Consider secondly the effect of injection of pituitary extract on the water exchanges, under the 3 sets of conditions: *a*) hypotonic, *b*) isotonic and *c*) hypertonic.

Hypotonic. With frogs in water, pituitary extract causes a rapid increase in body weight, up to 20 per cent in 5 hours. The initial rate of weight gain is about 10 per cent per hour, approximately five times the normal rate of flow of water through the frog. The magnitude of the increase in flow due to pituitary extract could not be due to oliguria alone, since it greatly exceeds normal urine output. The extract therefore decreases the resistance of the surface of the frog to the influx of water.

A comparison of figures 1 and 2 showing the effect of pituitary extract demonstrates

two phenomena. First, as noted above, it causes a greatly increased uptake of water, which exceeds normal urinary output by some five times. Secondly, since in the first 3 hours the effect of pituitrin was approximately the same in normal frogs and those with anal ligatures, it appears that this drug completely inhibits urinary water loss in the doses used. Pituitrin in effect prevents water loss through urine. This oliguric effect is probably renal in origin, because pituitary extract causes a marked reduction in the number of active glomeruli in the frog kidney, and consequent anuria (15). It seems that the uptake of water after pituitary extract is due to a combination of decreased resistance to the influx of water, and decreased excretion of urine.

Isotonic. In isotonic solution, pituitary extract delays the excretion of urine (fig. 3) but has no effect on the body weight when the escape of urine is prevented (fig. 4). An osmotic gradient favoring influx of water is necessary for the effect of pituitary extract to become apparent.

Hypertonic. When the osmotic gradient is reversed, pituitary extract has no effect on the already high rate of outflow of water through the skin.

SUMMARY

The frog offers a resistance to the influx of water from hypotonic solution, as compared to the outflow of water when the osmotic gradient is reversed. This resistance is greatly diminished by pituitary extract (posterior lobe). An oliguria is also produced by mammalian posterior pituitary extract, which contributes to the net gain in weight by water uptake from hypotonic solution.

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EFFECT OF THE BLOOD GLUCOSE LEVEL ON THE SECRETION OF THE ADRENAL CORTEX

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INVESTIGATIONS in recent years have shown that the adrenal cortex plays an important rôle in maintaining the resistance of the animal organism to stress (1, 2). In view of this, any study which contributes to an understanding of the mechanism which regulates the secretion of this endocrine organ assumes significance. It is now generally accepted that the activity of the adrenal cortex is controlled by the adrenocorticotrophic principle, which is secreted by the anterior pituitary.¹ The mechanism of the secretion of the adrenal cortex is; therefore, mainly associated with the factors influencing the release of the adrenocorticotrophic hormone from the anterior pituitary. Recently, it has been found that epinephrine plays an important rôle in the control of adrenal cortical activity and that this control is mediated through the anterior pituitary by bringing about a release of the adrenocorticotrophic hormone (1, 2). The manner by which epinephrine produces this effect is not known.

Since epinephrine and the blood glucose level are so closely interrelated, experiments have been performed to determine whether the blood glucose level *per se* would influence the activity of the adrenal cortex. Alterations in the cholesterol content of the adrenals have been used as a measure of elaboration of cortical hormones by the adrenal cortex. Evidence has recently been obtained that a relationship exists between the amount of cholesterol, present in the adrenal, with the gland's secretory activity (1, 2). The nature and character of this relationship are not well understood. The changes in the cholesterol content of the adrenal induced by hyperglycemia or hypoglycemia are presented.

PROCEDURE

White adult male rats of the Wistar and Sprague-Dawley strains, weighing between 225 and 280 grams were employed. The animals were starved overnight but allowed water.

Oral Administration of Glucose. The rats were intubated with a catheter and 2 ml. of a 50 per cent glucose solution were introduced orally by means of a hypodermic syringe into the experimental and 2 ml. of distilled water into the control animals. After certain time intervals, the animals were killed by the guillotine method. The adrenal glands were immediately dissected, weighed and analyzed for cholesterol according to the method of Schoenheimer and Sperry (4). The total cholesterol was

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¹ Deane, Shaw and Greep have presented evidence that at least in the rat the secretion of the electrolyte-regulating hormones of the adrenal gland is not under the control of the anterior pituitary (3).

determined in each case and the amount of cholesterol is reported as mg./100 mg. of fresh wet adrenal tissue. Blood sugar determinations were carried out according to the procedure of Somogyi (5).

It was found that handling and intubation of the animals led to an 'alarm' reaction, as indicated by a decrease in adrenal cholesterol. For this reason, it was essential to accustom the animals to the experimental conditions before being used. This was done by passing a fine rubber catheter into the stomach twice daily for at least eight days. Only healthy, growing rats are suitable for studies of adrenal cholesterol changes inasmuch as inanition or infection will markedly influence the adrenal cholesterol levels. The animals were kept as much as possible in an environment of 24° to 26° C.

Administration of Insulin. The experimental animals were injected subcutaneously with 0.5 I.U. of insulin while the control animals were injected subcutaneously

TABLE 1. CHANGES IN THE CHOLESTEROL CONTENT OF THE ADRENALS AND IN THE BLOOD SUGAR LEVELS IN NORMAL RATS AFTER ORAL ADMINISTRATION OF 2 ML. OF 50% GLUCOSE SOLUTION

NO. OF ANIMALS	TYPE OF EXPERIMENT	TIME OF DEATH AFTER INJECTION	WT. OF FRESH ADRENAL GLANDS	CHOLESTEROL CONTENT OF 100 MG. OF FRESH ADRENAL TISSUE	BLOOD SUGAR LEVEL IN MG. % AT TERMINATION
		<i>min.</i>	<i>mg.</i>	<i>mg.</i>	
18	Control 2 ml. H ₂ O	1	35	3.44 ± 0.10	72
12	Glucose	30	33	4.91 ± 0.16	81
12	Glucose	60	36	3.38 ± 0.18	99
12	Glucose	120	36	3.83 ± 0.11	90
7	Glucose	240	34	3.57 ± 0.09	
8	Glucose	300	36	3.21 ± 0.11	70
4	Glucose	360	34	3.70 ± 0.07	

¹ The animals were killed at the same time intervals as the glucose injected rats.

with 0.25 ml. of saline solution. The adrenal cholesterol and blood glucose levels were determined ninety minutes after administration.

RESULTS AND DISCUSSION

Table 1 illustrates the mean cholesterol changes in the adrenals as well as the mean blood sugar changes determined simultaneously in rats after oral administration of 2 ml. of a 50 per cent glucose solution in water. Oral administration of glucose produced a distinct increase in the adrenal cholesterol and an elevation of the blood sugar level half an hour after administration. Between 30 to 60 minutes, there is a sharp decline in the adrenal cholesterol content to normal levels. The increase in the adrenal cholesterol level, observed at thirty minutes, was significant inasmuch as the lowest values for cholesterol found at that time were definitely greater than the highest values obtained in the control animals. The rapidity of the rise and decline of the adrenal cholesterol after the administration of glucose is rather surprising.

Elmadjian, Freeman and Pincus (6) found that oral administration of glucose (1 ml. of a 50% solution) to rats produced a lymphocytopenia within one to two hours

after administration. We also observed a decrease in lymphocytes after oral glucose administration at a time at which the cholesterol levels of the adrenals were normal. The fall in lymphocytes, which is supposed to indicate a stimulation of the adrenal cortex, may possibly be due to the actual outpouring of adrenocortical hormones occurring within 30 to 60 minutes after glucose administration. The decline in adrenal cholesterol and the release of adrenocortical hormones at a time during which there still exists a pronounced hyperglycemia may be explained as follows.

a) The hyperglycemia causes a stimulation of the secretion of insulin, which according to Vogt (7), may directly stimulate the release of the adrenocorticotrophic hormone from the anterior pituitary.

b) The inhibition of the adrenal cortex, as indicated by the increased adrenal cholesterol content, leads to a lowered concentration of adrenocortical hormones in the blood which, according to Sayers and Sayers (8), causes a stimulation of the liberation of adrenocorticotrophic hormone from the anterior pituitary.

TABLE 2. MEAN CHANGES IN THE CHOLESTEROL CONTENT OF THE ADRENALS AND IN BLOOD SUGAR LEVELS IN NORMAL RATS AFTER SUBCUTANEOUS INJECTION OF 0.5 I.U. INSULIN

NO. OF ANIMALS	TYPE OF EXPERIMENT	TIME OF DEATH AFTER INJECTION	WT. OF FRESH ADRENAL GLANDS	CHOLESTEROL CONTENT OF 100 MG. OF FRESH ADRENAL TISSUE	BLOOD SUGAR LEVEL IN MO. % AT TERMINATION
		<i>min.</i>	<i>mg.</i>	<i>mg.</i>	
5	Control Uninjected	90	35	3.82 ± 0.03	72
12	Control 0.25 ml. Normal saline	90	32	3.59 ± 0.19	66
18	Insulin	90	32	2.04 ± 0.11	20

Abelin (9) observed that, 7 to 8 hours after oral glucose administration (1 gm/100 gm. of body weight) to rats, a reduction of about 25 per cent in the adrenal cholesterol occurred. In repeating Abelin's experiments, similar results were obtained. The adrenal cholesterol content apparently increased within a short period after oral administration of glucose, then rapidly fell to a normal level while hyperglycemia still persisted and stayed at a normal level for several hours, during which time the blood sugar level gradually became normal. After 8 hours, a lowering in the adrenal cholesterol content was observed.

Since the initial hyperglycemia led to an increase of the adrenal cholesterol content, investigation was directed toward the determination whether hypoglycemia would effect a lowering of the adrenal cholesterol.

As can be seen from table 2, insulin-induced hypoglycemia effected a stimulation of the adrenal cortex as indicated by the lowered adrenal cholesterol level. This finding is in agreement with that of Gershberg and Long (10) who observed a fall in the adrenal ascorbic acid and with that of Vogt (7) who found a depletion in the adrenal lipids of rats following insulin injection.

The findings reported in this paper show that hyperglycemia produced by oral glucose administration leads to an inhibition of the release of the hormones from the

adrenal cortex, as indicated by an increase in the adrenal cholesterol content. The data given support the assumption that the initial hyperglycemia leads to a diminished release of epinephrine by the adrenal medulla with a correspondingly lessened release of the adrenocorticotrophic principle from the anterior pituitary. In hypoglycemia, the reverse takes place. Whether or not this effect of the blood glucose level on the secretion of the adrenal cortex is directly mediated through the anterior pituitary or indirectly through the adrenal medulla is not as yet established.

SUMMARY

Hyperglycemia induced by oral administration of glucose in rats causes, within 30 minutes, an elevation of the adrenal cholesterol level indicating an inhibition of the adrenal cortex. Hypoglycemia induced by insulin injection in rats produces a decrease of the adrenal cholesterol level 90 minutes after administration indicating a stimulation of the adrenal cortex.

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ACTION OF VITAMIN P ON THE STABILITY OF CONNECTIVE TISSUE GROUND SUBSTANCE¹

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WHEN vitamin P was discovered, its function was said to be the regulation of the permeability and strength of capillaries (1). Since then, all the work which has been done in this field has been focused on capillaries, and the possibility that this vitamin might be concerned with the control of the permeability of other tissues has received scant attention.

In a previous communication (2), the author showed that rutin, a flavonol type of vitamin P, inhibited the diffusion of testicular hyaluronidase and control saline solutions injected intradermally in rats. Since the spread of the control solution could conceivably be associated with the activation of endogenous hyaluronidase, no conclusions could then be made as to whether rutin acted directly by inhibiting hyaluronidase, or indirectly by some action on the substrate. The probability that the latter was the case was suggested to the author by the findings of Griffith and Lindauer (3), that rutin prevented cerebral hemorrhage in hypertensive patients with increased capillary fragility. Although their study was stimulated by Pater-son's (4) suggestion that cerebral hemorrhage developed on the basis of rupture of intimal capillaries, such a mechanism has never been clearly demonstrated in this type of vascular accident. It is more likely that cerebral hemorrhage is due to direct rupture of the whole thickness of the artery wall as a consequence of arterio-sclerotic weakening combined with increased arterial blood pressure. This suggests that rutin acts on the entire vascular apparatus rather than solely on the capillaries. Gorev and Smirnova-Zamkova (5) have shown that the pericapillary sheath, the media and externa of arteries, and the connective tissue in general, all have in common, argyrophilic ground substance. It therefore appeared that the strengthening effect of rutin on the capillaries and arteries might be referable to a stabilizing effect on the ground substance which they contain. If such were the case, the stabilization of the generalized system of ground substance by rutin should be non-specific and demonstrable against spreading agents other than hyaluronidase. To check this hypothesis, rutin was tested against a potent, long-acting, synthetic spreading factor, azoserum. The studies were then extended to other types of vitamin P.

PROCEDURE

A 6.5 per cent solution of rutin³ was prepared in 0.2N sodium hydroxide. The pH , as measured in the Beckman meter, was 9.4. On the addition of 1.25 cc. N

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² Fellow in Medical Sciences, American National Research Council.

³ Furnished by the F. W. Horner Co., courtesy Dr. L. Mitchell and Mr. G. Ling.

hydrochloric acid to 20 cc. rutin solution, a creamy emulsion formed whose pH was 8.4. A control sodium hydroxide solution of similar pH was also prepared. The other types of vitamin P, being water soluble, were injected as aqueous solutions. All injections of vitamin P were intraperitoneal, and preceded the spreading factors by 40 minutes to 4 hours.

The spreading solutions consisted of azoserum (6) as prepared, and testicular hyaluronidase⁴ (2 mg. per injection), and physiological saline. They were colored by adding one third of their volume of doubly filtered Higgins india ink. The injection mass in each case was 0.2 cc. The active spreaders were injected into the right flank, the control saline into the left. An attempt was made to place all injections at the same relative point in the flanks, midway between the insertion of the upper and lower extremities, and 4 cm. from the mid-dorsal line. If an appreciable amount of the injection leaked out superficially or went too deeply, the animal was immediately killed and discarded. Twenty hours after the intradermal injections, the animals were killed and skinned. The skins were mounted with the inner side up. The inked areas, which were clearly delineated, were outlined and transposed onto cellophane paper and the enclosed areas measured in square inches by a planimeter. The results were analyzed by comparing the difference between means with the standard error of the difference, and by the method of Analysis of Variance. Reduction of spreading by less than 15 per cent was arbitrarily considered to be insignificant.

OBSERVATIONS

Effect of Rutin on Diffusion of Azoserum. Rutin administered to rats⁵ in single doses of 65 mg. (1.0 cc.) 4 hours before the spreading factor, resulted in inhibition of the diffusion of both the azoserum and the control saline to a degree which was highly significant statistically (table 1). Inspection of the means reveals that the control pH solution failed to exert any significant effect.

Effects of Hesperedin Methyl Chalcone⁶ and Epicatechin^{6,7} on Diffusion of Hyaluronidase. One hour before the spreading factor was injected into the rats⁸ the above types of vitamin P were given in doses of 100 mg. (1.0 cc.) and 10 mg. (0.25 cc.) respectively. Diminution in the areas of spread of both hyaluronidase and control saline solutions failed to occur. Negative results were also observed in guinea pigs when the spreading factor was injected 40 minutes after the second of two 5 mg. doses of epicatechin given 6 hours apart. The guinea pigs were used in addition to rats since the vitamin P activity of catechins was first described in this species. Epicatechin was used in a small dosage because it was claimed to be the most potent type of vitamin P (7). Possibly larger doses might have been effective.

Effects of Multiple Injections of Esculin on Diffusion of Azoserum and Hyaluronidase. The results in *experiment 2* suggested that some types of vitamin P might require extended administration before their effects became apparent. Single

⁴ Purchased from the Tremond Co., N. Y. The potency was listed as 100 T.R.U./mg.

⁵ Male albinos, 275-325 gm.

⁶ Furnished by the Hoffman-Laroche Co., courtesy Mr. P. Blanc.

⁷ The catechin was a mixture of epimers containing 35 per cent epicatechin.

⁸ Female albinos, 225-300 gm.

TABLE 1. EFFECTS OF RUTIN ON THE AREAS OF SPREAD¹ OF INKED AZOPROTEIN AND OF CONTROL SALINE SOLUTIONS

STRAIGHT CONTROL		NaOH CONTROL		RUTIN IN NaOH	
Azoprot.	Saline	Azoprot.	Saline	Azoprot.	Saline
3.86	0.60	3.40	0.80	1.90	0.30
2.60	0.80	3.40	0.70	1.80	0.40
4.00	0.53	3.20	0.64	4.00	0.50
3.50	0.70	3.85	0.55	1.76	0.40
3.50	0.60	3.00	0.65	2.63	0.45
3.05	0.60	2.35	0.40	1.93	0.30
3.83	0.75	4.00	0.60	2.57	0.43
2.72	0.60	2.96	0.70	1.65	0.44
2.94	0.70	3.45	0.65	1.84	0.45
4.20	0.68	4.30	0.70	2.34	0.43
3.02	0.69	4.20	0.50	2.07	0.30
2.91	0.58	3.77	0.64	1.50	0.40
3.35	0.83	3.41	0.60	1.92	0.30
3.20	0.70			1.62	0.23
4.93	0.70			1.60	0.33
				2.72	0.43
				1.82	0.44
\bar{X} 3.44	0.67	3.48	0.62	2.10	0.38

		AZOPROT.	SALINE
Mean difference		1.34 ± 0.22^2	0.29 ± 0.026^2
Variance ratio	Obs.	30.0 ²	104.0 ²
	0.01 sig.	7.56	7.56

¹ In square inches. ² Highly significant.TABLE 2. EFFECTS OF ESCULIN ON THE DIFFUSION OF HYALURONIDASE, AZOPROTEIN, AND SALINE¹

	CONTROLS			ESCULIN		
	H'ase	Azoprot.	Sal.	H'ase	Azoprot.	Sal.
Range	1.2-3.0	2.17-3.72	0.4-0.8	1.23-3.5	1.84-3.1	0.20-0.63
N	18	15	33	18	15	33
\bar{X}	2.23	2.96	0.548	1.69	2.38	0.465
			H'ase	Azoprot.	Saline	
Mean difference			0.54 ± 0.19^2	0.58 ± 0.17^3	0.083 ± 0.025^3	
Variance ratio	Obs.		10.2 ³	12.32 ³	11.1 ³	
	0.01 sig.		7.44	7.68	7.04	

5 mg. doses of esculin⁹ (1.0 cc.) were given to rats¹⁰ daily for 6 days, prior to the spreading factors. The subsequent diffusions of azoserum, hyaluronidase and control saline solution were all inhibited to a highly significant degree (table 2).

Effects of Vitamin P Deficiency on Permeability of Ground Substance. In the experiments listed so far, the various types of vitamin P were administered to animals on normal diets¹¹ and the results could be interpreted as pharmacological rather than vitaminic. It was therefore decided to determine if the state of tissue permeability was altered in vitamin P-deficient rats. Unfortunately, gray oats, one of the major constituents of a truly vitamin P-free diet (8), was not available locally so that this diet could not be used. As an alternative procedure, advantage was taken of Parrot's finding (9) that turnips contained a substance with the properties of antivitamin P. Two hundred gamma of an alcoholic extract of turnip were said to produce a drop in capillary resistance which could be prevented by 20 gamma of catechin. It was claimed that the turnip factor accelerated the *in vitro* oxidation of ascorbic acid, whereas vitamin P substances inhibited this reaction. Since the action of the turnip factor was opposed to that of vitamin P both *in vivo* and *in vitro*, it was called antivitamin P.

Forty-five of a group of 60 male white albino rats weighing from 180 to 210 gm. were put on an antivitamin P regime by maintaining them on a diet of turnips and water *ad lib.*, for 14 days. The remaining 15 rats served as normal diet controls. By the 12th day, 8 of the turnip group and 1 of the control group had died. At this time, the turnip group was divided into 3 sub-groups. One sub-group was given 50 mg. ascorbic acid intraperitoneally on the 12th and 13th days. Another sub-group was given 5.0 mg. esculin intraperitoneally on the same days, and the third sub-group was not treated. On the 14th day intradermal injections of 0.2 cc. inked azoprotein and saline were made in all animals and they were then treated as previously.

RESULTS

The most striking finding (table 3) was the marked increase in diffusion of both azoprotein and saline in the rats on the turnip diet. With the amount of esculin used, the increased spread of the azoprotein was moderately but significantly reduced. Esculin had no significant effect on the enhanced diffusion of saline. Ascorbic acid failed to inhibit the increased spread of both azoserum and saline.

DISCUSSION

The results indicate that at least 2 types of vitamin P, rutin and esculin, are capable of hindering the diffusion through connective tissue of enzymatic and non-enzymatic spreading factors as well as inert saline-ink mixtures. Since the retardation of diffusion appears to be non-specific, it is probable that vitamin P does not act directly on the substances injected into the skin but rather, it decreases the permeability of the connective tissue in a non-specific way. When vitamin P

⁹ Purchased from the Mercantile Import and Export Co., N.Y.

¹⁰ Hooded males, 150-200 gm.

¹¹ Purina Fox chow for rats. Various greens for guinea pigs.

deficiency was induced, the permeability of the connective tissue was markedly increased and this was partially reversed by esculin. It has been shown that hyaluronidase spreads through isolated dead skin (10). Its spread must therefore be largely independent of the circulation and must occur directly through the connective tissue ground substance. Thus vitamin P appears to be one of the factors which governs the strength and permeability of ground substance. As was mentioned previously, Gorev and Smirnova-Zamkova (5) have shown that the ground substance present in connective tissue is only part of a diffuse system of this material and that it is also present in the media and adventitia of arteries. Bensley (11) has also identified it in the intima of arteries. Thus by acting on the ground sub-

TABLE 3. EFFECTS OF ESCULIN AND OF ASCORBIC ACID ON THE INCREASED DIFFUSION OF AZOPROTEIN AND SALINE IN RATS FED TURNIPS (ANTIVITAMIN P)

	REGULAR DIET		TURNIP DIET		TURNIP DIET + ESCULIN		TURNIP DIET + ASCORBIC ACID	
	Azop.	Sal.	Azop.	Sal.	Azop.	Sal.	Azop.	Sal.
Range.....	2.25-4.30	0.37-0.85	3.92-6.50	0.80-1.67	3.16-5.24	0.75-1.55	4.04-5.23	0.90-1.95
N.....	11	11	9	9	11	11	9	9
\bar{X}	3.32	0.54	5.07	1.24	4.23	1.00	4.45	1.18

		TURNIP DIET VS. REGULAR DIET		TURNIP DIET + ESCULIN VS. TURNIP DIET		TURNIP DIET + ASCORBIC ACID VS. TURNIP DIET	
		Azop.	Sal.	Azop.	Sal.	Azop.	Sal.
Mean difference		1.75 \pm 0.35 ³	0.70 \pm 0.11 ³	0.85 \pm 0.35 ²	0.24 \pm 0.125	0.62 \pm 0.32	0.06 \pm 0.14
Variance ratio	Obs.	25.6 ³	47.3 ³	5.8 ²	3.82	3.67	—
	0.01 sig.	8.28	8.28	4.4	4.4	4.49	—

¹ Figures in sq. inches. ² Significant. ³ Highly significant. ⁴ Below arbitrary level of significance.

stance of vascular walls, vitamin P appears to be a factor which determines the strength of vessel walls. Such an action on the 3 layers of the artery, rather than a capillary mechanism may account for the decreased incidence of cerebral hemorrhage which Griffith and Lindauer (3) observed following administration of rutin to patients with hypertension and increased capillary fragility.

The ability of vitamin P to increase capillary resistance has never been correlated with an action on any particular layer of the capillary wall. Several investigators (12-18) have shown that in pure scurvy uncomplicated by vitamin P deficiency, the capillary resistance is normal. Vitamin P is therefore able to act on a capillary layer which is not damaged in scurvy. This precludes the interendothelial cement whose production is said to be arrested in scurvy (19). Since the pericapillary sheath is believed to be a condensation of ordinary connective

tissue (19) the finding that vitamin P increases the resistance of connective tissue strongly suggests that the site of action of vitamin P on the capillary wall is the pericapillary layer.

The full significance of vitamin P deficiency on the body economy is as yet unknown, as very little investigative work has been carried out in this direction. Duran-Reynals (20) has reviewed abundant evidence which indicates that susceptibility to infection varies directly as the degree of permeability of connective tissue. In experimental arteriosclerosis, Duff (21) has shown that damage to vascular ground substance precedes deposition of lipoids. Gorev and Smirnova-Zamkova (5) maintain that in hypertension and arteriosclerosis, there are degenerative changes in the entire system of ground substance. In view of the role of vitamin P in maintaining the stability of ground substance, it is possible that the long term sequel of vitamin P deficiency is an increased susceptibility to infectious and degenerative processes.

SUMMARY

Rutin and esculin, two types of vitamin P, inhibited the spread of intradermally injected hyaluronidase, azoserum and saline. The results are believed to be due to a non-specific increase in the resistance of the connective tissue ground substance. In vitamin P deficiency, induced by feeding turnip antivitamin-P factor, the permeability of connective tissue was increased. This was partially reversed by the administration of esculin, but not by ascorbic acid.

In addition to demonstrating a wider site of action of vitamin P than hitherto recognized, the experimental findings add further evidence in favor of the existence of this vitamin. It is suggested that vitamin P regulates capillary resistance and permeability by an action on the pericapillary sheath. It is suggested that the weakening of ground substance by vitamin P deficiency might predispose to the development of degenerative and infectious diseases.

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POTENTIATION OF TWITCH TENSION AND PROLONGATION OF ACTION POTENTIAL INDUCED BY REDUCTION OF TEMPERATURE IN RAT AND FROG MUSCLE¹

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DURING the past four years it has been observed that the rising time and the peak tension of the twitch of the *in situ* rat triceps surae were slightly greater in experiments carried out in the winter than in those done in the summer. The room temperature was about 26° C. in the winter and about 31° C. in the summer. An effect of temperature changes on the modification of twitch response in the leg muscle suggested itself because it seemed likely that the temperature of anaesthetized animals may decrease more in the cooler room. No report on the effects of temperature changes on twitch tension of *in situ* mammalian muscle was found in the literature. However, it was found that an observation (1) on the effects of cooling on the isolated nerve-diaphragm preparation of the rat had been made. In the fatigued preparation cooling increased the rising time, tension and action potential duration in response to single indirect shocks. The increased duration of action potential produced by cooling skeletal muscle (2) is of interest in the present report because it has recently been shown (3) that the potentiation of tension and the increase of rising time of the twitch in rats given intraperitoneal injection of KCl are accompanied by an increase in duration of the action potential. The present study was begun in order to determine the changes in mechanical and electrical response of *in situ* rat muscle induced by lowering the temperature approximately 10° C. below normal. Discrepancies between our findings and the observations (4, 5) on isolated frog muscle suggested that experiments on frog muscle with intact circulation should be carried out. A preliminary report on this study was made before the American Physiological Society at the meeting in Minneapolis in September, 1948.

METHODS

Male rats, of the Anheuser-Busch strain, weighing approximately 275 gm. were anesthetized with 300 to 350 mg. of sodium barbital per kg. The experiments with the control group of rats were done at room temperature (31° C.). Sixteen rats were placed in a cold room (10° C.) until the rectal temperature was reduced to about 20° C. Preparation for recording was made after removal from the cold room in 8 animals and the preliminary portion of preparation was made before removal in 5 animals. Three rats were prepared and stimulated in the cold room. The muscle of 3 rats was cooled by placing the leg in a cooling chamber. The triceps surae was pre-

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pared for stimulation and recording as previously described (6). Action potentials were recorded from the gastrocnemius muscle with a cathode ray oscillograph, one lead electrode being placed in the belly and the other in the tendon of the muscle. The myograms were made with an isometric lever. Determinations of rat plasma K were made with a flame photometer designed by Dr. Theodore Weichselbaum and Dr. P. L. Varney.

The gastrocnemius muscle of the frog was cooled by placing the leg in Ringer's solution maintained at the desired temperature with a water bath, the hind legs being immobilized by section of the spinal cord at a level which did not impair breathing.

Some definitions and qualifications of terms employed in the presentation of the results and the discussion are given for purposes of clarification. The expression, membrane 'breakdown,' is used for convenience in reference to changes in the fiber membrane during the passage of the excitation wave. The twitch is the single response of all component muscle fibers to a single indirect shock, the shock strength being 3 to 4 times maximal. The term potentiation designates an increase of twitch

TABLE 1. AVERAGE VALUES FOR THE PEAK TENSION AND TIME COURSE OF THE TWITCH IN THE IN SITU TRICEPS SURAE OF THE RAT WHICH SHOW THE MODIFICATIONS INDUCED BY REFRIGERATION OF THE ANIMAL AT 10°C.

NO. OF RATS	GROUP NO.	ROOM TEMPERATURE	RECTAL TEMPERATURE	TWITCH TENSION	RIISING TIME	TIME OF HALF FALL	TIME OF HALF FALL/RIISING TIME
		°C.	°C.	gm./gm. muscle	msec.	msec.	
8	1	31	26.1 ± 0.52 ¹	434 ± 9.6	35.6 ± 2.37	38.1 ± 4.14	1.06 ± 0.04
5	2	26	22.0 ± 0.35	523 ± 6.8	74.2 ± 1.02	92.8 ± 3.7	1.24 ± 0.03
4	3 ²	31	36.4 ± 0.34	270 ± 10.4	14.2 ± 1.05	21.5 ± 1.19	1.57 ± 0.04

¹ Standard error of the mean = $\sqrt{\frac{\sum d^2}{n(n-1)}}$

² Control group given no refrigeration.

tension resulting from increased contraction of the component fibers. Tension is expressed in gm./gm. of fresh muscle; the measurements as cited in this paper refer to developed tension. The resting tension was usually approximately 75 gm./gm. of muscle. The rising time was measured from the beginning of the upward deflection of the record to the peak. The time of half fall was measured from the peak of the tension curve to the point where this curve has returned half way to the initial resting tension.

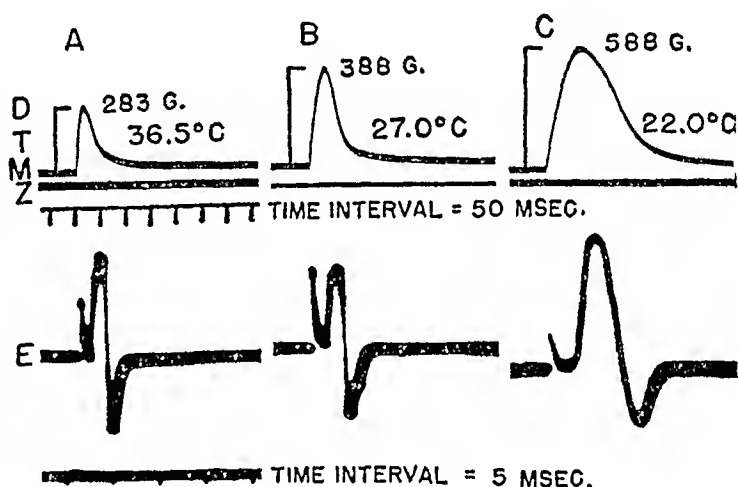
RESULTS

Observations on the Rat. The rats in group 1 of table 1 were removed from the refrigerator when the rectal temperature had fallen to about 21° C. and prepared for stimulation at a room temperature approximately 31° C.; these experiments were carried out in August. The records were obtained by the time the rectal temperature had risen to 25° C. to 29° C. A comparison of average values in this group of animals with those obtained in control rats (group 3) shows a 61 per cent increase of peak tension, a marked increase (151%) of rising time and a smaller increase (77%) in time of half fall in the twitch response of the cooled animals. The rats in group 2 were par-

tially prepared for stimulation before removal from the refrigerator and the records were obtained by the time the rectal temperature had increased to about 22°C . at a room temperature approximately 26°C .; these experiments were carried out in November. Under these conditions 94 per cent, 423 per cent and 331 per cent increases, respectively, of tension, rising time and time of half fall were induced. The rising phase of the twitch was prolonged more than the falling phase, this difference being more pronounced with moderate cooling (cf. *group 1* with *group 2* in table 1 and *B* with *C* in fig. 1). The myogram in figure 1*B* shows the least potentiation of twitch tension obtained in *group 1* and the myogram in figure 1*C* shows the greatest potentiation obtained in *group 2* of table 1. These myograms illustrate the progressive slowing of the twitch response by cooling.

The marked difference in duration of twitch response in *groups 1* and *2* of table 1 suggested that the difference in muscle temperature may be greater than that indi-

FIG. 1. EFFECTS OF REFRIGERATION of the rat on the triceps surae mechanical response and gastrocnemius action potential, the muscles being stimulated indirectly with single shocks. *D*: Developed tension expressed in gm/gm. of fresh muscle. The resting tension was approximately equal for the records shown in this fig. *T*: rectal temperature at the time the records were made; *M*: myograms; *Z*: zero tension; *E*: action potentials; *A*: records made 2 hours after giving sodium barbital; *B*: records made after 1 hour of refrigeration at 10°C .; *C*: records made after 2.5 hours of refrigeration at 10°C . The action potential record in *C* was retouched.



cated by the rectal temperature. Because the animals in *group 1* were prepared for stimulation in a warm room (31°C .) it seemed likely that the muscle temperature may have been much higher than the rectal temperature at the time of recording. In order to follow more closely the temperature changes in the muscle the stimulating and recording apparatus was placed in the refrigerator. In a warm room a series of 3 rats was prepared for stimulation and a small thermometer was placed beside the contralateral gastrocnemius muscle through a small incision near the heel. The animals were then placed in the refrigerator for periodic stimulation and recording. The results of one of these experiments are shown in figure 2. The peak twitch tension increased sharply as the muscle temperature decreased from 37° to 30°C ., and less rapidly as the temperature fell from 30°C . to 24°C . The decline in rate of potentiation of tension in the lower range of temperature was accompanied by a marked increase in duration of the twitch response. The sharp increase of tension observed in the higher range of temperature was accompanied by a less marked increase in duration of the twitch response. The effects obtained by cooling the muscle gradu-

ally disappeared as the muscle was allowed to return to normal body temperature. At 37° C. the twitch duration was normal and peak twitch tension was slightly above normal. The results obtained during the progress of refrigeration (fig. 2) indicate that the muscle temperature was about 30° C. in *group 1* of table 1 and about 24° C. in *group 2*.

The effects of cooling on the action potential are of particular interest in the experiments done during the progress of refrigeration because it was possible to observe the changes at different temperatures with the recording electrodes in the same position. The duration of the action potential increased slowly at first and then more abruptly as the muscle temperature fell below 32° C. (fig. 2). The height of the action potential increased initially but it decreased toward the normal height as the muscle temperature fell below 29° C. The increase in duration of the action potential is very similar to the increase observed during the progress of twitch potentiation induced by intraperitoneal injection of KCl in the rat (3). It should be noted that the height and duration of the action potential were normal when the muscle was allowed to return to normal body temperature.

The muscle was cooled in 3 rats by placing the leg in a cool chamber (17° C.-20° C.) for about 20 minutes. This treatment brought about a 37 ± 4 per cent increase of peak tension, a 172 ± 7 per cent increase in rising time, a 308 ± 25 per cent increase in time of half fall and a 253 ± 34 per cent increase in duration of the action potential. Records from one of these experiments are shown in figure 3. Although the action potential height was lower in 2 of the 3 experiments at the time maximum potentiation was attained, the height increased initially in all 3 experiments.

Effects of Local Cooling in the Frog. The results obtained by cooling the *in situ* gastrocnemius in 3 summer frogs with intact circulation and normal respiration were similar to those obtained in the rat by moderate refrigeration. The average high temperature of the Ringer's solution bath in which the leg was immersed was 27.5° C.; the average low temperature was 14.5° C. Change of the temperature from the high to the low level produced a 40 ± 9 per cent increase of peak tension, a 200 ± 8 per cent increase of rising time and a 154 ± 30 per cent increase in time of half fall in the twitch response. The records in figure 4 show typical effects of the extreme temperatures on mechanical and electrical responses. Records taken at intermediate temperatures showed intermediate modifications of twitch tension and time course and of action potential duration.

Similar cooling of isolated summer and winter frog gastrocnemii produced varied effects on twitch tension. In 5 experiments the peak tension was decreased and in one it was increased by reduction of the temperature of the bath. The slowing of the twitch was about the same as that observed in the cooled intact frog muscle. Similar results were obtained in *in situ* muscle of winter frogs; cooling induced a slight decrease of gastrocnemius twitch tension in 3 and a small increase in 1 of 4 experiments.

Since the plasma K is markedly increased in rats showing potentiation of twitch tension after KCl treatment (3) the possibility that a shift of K to the extracellular fluid may be responsible for the potentiation of twitch tension in cooled muscle was explored. The average values for analyses of plasma K were 4.85 mM per liter in 5 rats cooled to 20° C. and 3.99 mM per liter in 3 control animals.

DISCUSSION

It seems unlikely that the 24 per cent increase of plasma K found in rats cooled to 20° C. plays a major rôle in bringing about the 94 per cent increase of twitch tension in these animals, because a previous study (3) has shown that a 200 per cent in-

FIG. 2. DIAGRAM SHOWING CHANGES IN RESPONSE of the rat triceps surae to single indirect shocks as the muscle temperature was reduced from 37°C. to 24.5°C. and then increased to 37°C. The muscle was cooled by placing the anesthetized animal in a cold room (10°C.). The intervals between the recordings were 10 minutes in all cases except the first, which was 5 minutes, and the last, which was 75 minutes. The peak tension of the twitch is expressed as gm/gm. of fresh muscle. The recorded action potential height is given in mm.

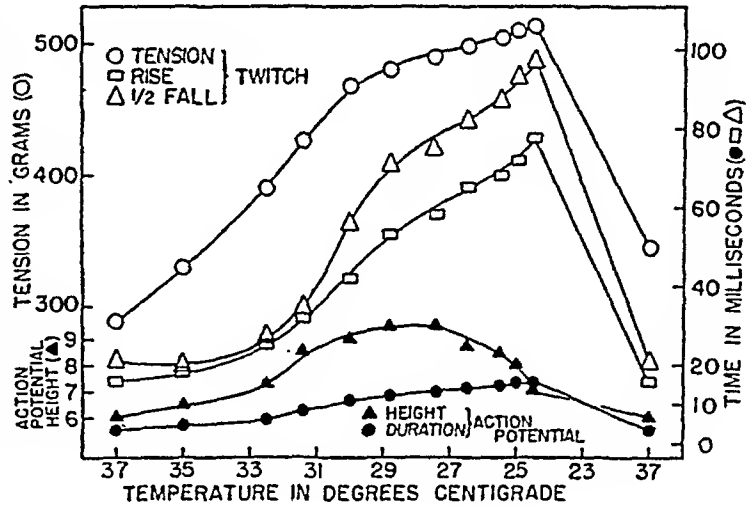


FIG. 3. EFFECTS OF COOLING THE LEG in a chamber on the response of the rat triceps surae to single indirect shocks. D: developed tension expressed in gm/gm. of fresh muscle; T, A: rectal temperature before local cooling; B: temperature of the cooling chamber 25 minutes after the leg was inserted; C: rectal temperature after local cooling. M: myograms; Z: zero tension; E: action potentials.

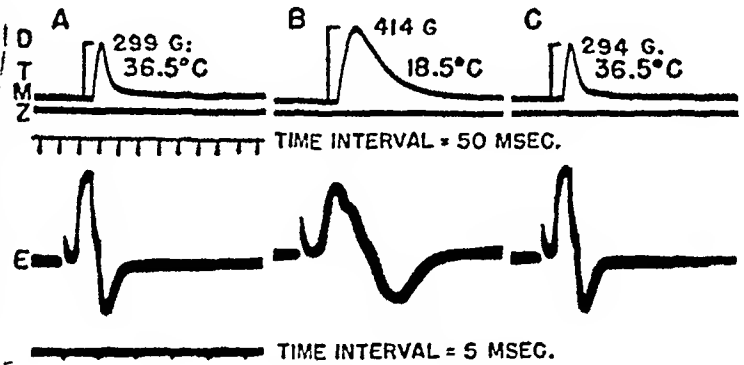
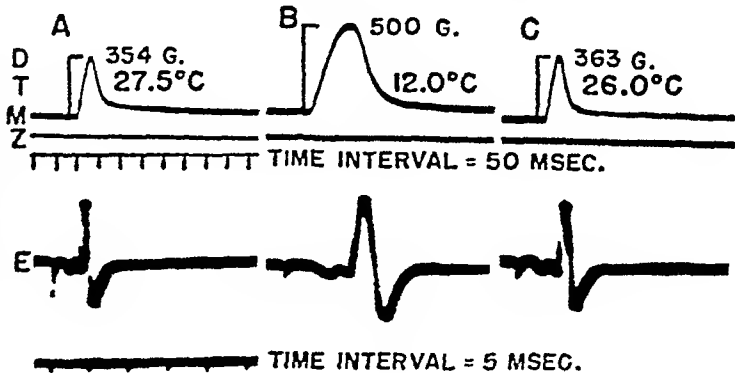


FIG. 4. EFFECTS OF COOLING THE LEG in a Ringer's solution bath on the response of the frog gastrocnemius to single indirect shocks. D: developed tension expressed in gm/gm. of fresh muscle; T: temperature of the bath; M: myograms; Z: zero tension; E: action potentials.



crease of plasma K concentration resulting from intraperitoneal injection of KCl produces only a 30 per cent increase of twitch tension. It should be noted, however, that both K treatment and cooling produce prolongation of the action potential and an increase of the ratio of rising time to falling time in the potentiated response of rat muscle to single indirect shocks.

Findings on the effect of temperature on the response of isolated frog muscle to single shocks are contradictory. For example, Gad and Heymans (4) reported that

a reduction of temperature from 30° C. to 19° C. induced a decrease of tension development, while Bernstein (5) found that similar reduction of temperature may either increase or decrease tension development in response to single shocks. Other reports in the literature agree with the findings of Gad and Heymans. The observation in this study that reduction of temperature from 27.5° C. to 14.5° C. increased twitch tension of *in situ* muscle in summer frogs, but usually decreased twitch tension of *in situ* muscle in winter frogs and of isolated muscle from summer and winter frogs, suggests that muscle response to temperature changes depends upon the nutritional state of the muscle. The responsible factors are not yet recognized. The potentiation of twitch tension obtained by local cooling in the rat and in the frog rule out the possibility that the effects of cooling on muscle response are due to metabolic changes induced by refrigeration of the whole animal.

The suggestion in connection with studies on the effects of KCl treatment, that increased duration of action potential as recorded from a multifibered muscle may be due in part to increased duration of membrane 'breakdown,' is supported by observations on the effects of cooling. Sanderson (2) found that action potential duration is increased and that conduction rate is reduced in the frog sartorius muscle by cooling. On the basis of his observations he suggested that cooling increases the duration of potential disturbance at a given point during the passage of an excitation wave. From the data recently reported (7) on the effect of temperature changes on the conduction rate and spike duration in single motor nerve fibers of the toad it is possible to calculate the change in length of the excitation wave during the passage of an impulse. The wave length was approximately doubled by reduction of the temperature from 25° C. to 5° C. The duration of the action potential was increased about 5 times by the same change of temperature. In so far as the findings for nerve fibers are applicable to muscle fibers these data support the view that duration of membrane 'breakdown' is greater in a cool than in a warm muscle fiber during the passage of an excitation wave. Whether there is a causal relation between increased duration of membrane 'breakdown' and increased contraction of muscle fibers, capable of responding with such increase of tension, cannot be decided with certainty. The view that such a causal relation exists is consistent with the findings on the rat and on the *in situ* summer frog muscle, and can be reconciled with the findings on the winter frog muscle and on isolated frog muscle by making the assumption that such muscles are incapable of responding with an increased tension.

SUMMARY

Observations were made on the modification of twitch response induced by cooling the rat triceps surae *in situ* and the frog gastrocnemius both *in situ* and isolated. The rat muscles were cooled by refrigeration of the whole animal or by placing the leg in a cooling chamber. The frog muscles were cooled by placing the leg or the isolated muscle in cool Ringer's solution.

Cooling the rat triceps surae from 37° C. to 24° C. by refrigeration increased twitch tension about 80 to 90 per cent and increased the duration of the action potential about 300 per cent. The time course of the twitch was increased about 350 per cent. The rising phase of the twitch was prolonged more than the falling phase, this

difference being more pronounced in the case of moderate cooling. Reduction of the temperature of the bathing fluid from 27.5° C. to 14.5° C. induced changes in the twitch response and action potential of the *in situ* gastrocnemius muscle of summer frogs similar to those observed in rat muscle. The same change of temperature usually produced a decrease of twitch tension of *in situ* muscle of winter frogs and of isolated frog muscle.

Although the effect of cooling in the rat apparently is not due to a change in concentration of plasma K, moderate cooling and K treatment induce similar changes in time course and tension of the twitch and in duration of the action potential. Support is offered for the view that the duration of membrane 'breakdown' produced by the passage of an excitation wave along the muscle fiber is prolonged by cooling. A causal relation between increased duration of membrane 'breakdown' and increased contraction of muscle fibers is suggested.

The author is indebted to Dr. W. A. Quebedeaux for technical assistance.

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OXYGEN CONSUMPTION AND COOLING RATES IN IMMERSION HYPOTHERMIA IN THE DOG¹

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MANY observations have been made on the effect of reduced body temperature on oxygen consumption. Overall conclusions are made difficult, however, by the wide range of experiment designs. Whether anesthesia was used, if so, depth and type, method of inducing hypothermia, duration of exposure, and species variation are but a few of the many factors tending to preclude generalizations. Too, variation in shivering response within a given set of experiments must be considered.

While the rate of cooling of the intact organism has received considerably less critical analysis, it likewise is subject to the same variables mentioned above. In an effort to derive a clear picture of the response of the hypothermic dog a series of experiments was designed wherein the procedure was constant. From these experiments it is apparent that shivering, which is in large measure an individual characteristic (at least under anesthesia), has a profound influence on oxygen consumption and rate of cooling. Only at the lowest extremes of body temperature, below which shivering is never seen, is it possible to predict accurately the metabolic response of a given animal.

METHODS

Fifteen experiments were conducted on 7 unselected but generally healthy mongrel dogs. On two occasions, separated by about 10 days, each dog was cooled to a rectal temperature of approximately 20°C. and rewarmed. In one case the dog was cooled a third time, using a longer lasting anesthetic.

After being fasted 24 hours the undepilated² dog was anesthetized with sodium pentothal³ *per venam* and secured in a supine position to a dog board. An endotracheal tube and rectal thermocouple were inserted and duplicate O₂ consumption determinations were made using a Sanborn respirometer. The preparation was then lowered into a tub of iced water (2°-4°C.) in a position about 10° from the horizontal. Immersion was complete except for the head, neck and ventral portion of the chest. The O₂ consumption was recorded continuously and the rectal and bath temperatures every 24 seconds.⁴ Pulse rates and shivering were observed and noted by a recorder. Additional pentothal was supplied as indicated to keep the anesthesia just adequate. The average total pentothal dose was 32 mg/kg. body weight, given in 5 per cent solution. In only one instance was more pentothal required at a rectal temperature below 35°C., the cold effect apparently having sufficiently augmented the anesthesia by this time to keep the dog quiet.

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² A series of trials by Haterius (3) and an observation by Spealman (2) failed to reveal any influence of depilation on rate of cooling in the dog.

³ Kindly donated by Abbott Laboratories, Inc.

⁴ 'Speedomax' recorder, Leeds & Northrup Co., Philadelphia.

At a rectal temperature of 20°C. the preparation was removed from the cold bath and rewarming measures at once instituted. These measures took the form of spraying with water of 40° to 42°C. for about 5 minutes following which the dog was left in room air of 25° to 28°C. to complete the rewarming, or immersion in a tub of water at 40°C. throughout the temperature ascent, or combinations of immersion in warm water and exposure to room air. With the return of consciousness and motor activity the measurements were terminated.

RESULTS

Oxygen Consumption during Cooling. The O₂ consumption before immersion and at rectal temperatures of 35°, 30°, 25° and 20°C. are presented in table 1.

TABLE 1. OXYGEN CONSUMPTION OF DOGS, CC/KG/MIN. (STPD), DURING COOLING BY IMMERSION IN COLD WATER

DOG	EXPER.	WT. KG.	38-39°	35°	30°	25°	20°
1	A	9.4	6.9	8.2	10.3	6.0	1.1
	B	7.9	6.3	4.8	4.2	4.6	1.0
2	A	13.6	6.3	4.2	5.7	5.1	1.6
	B	14.0	7.3	5.9	4.7	4.8	1.9
3	A	15.6	5.0	5.0	4.9	2.5	2.2
	B	14.0	6.0	4.4	4.8	3.5	1.0
4	A	14.0	6.2	6.0	11.5	8.4	1.9
	B	12.8	5.8	12.5	18.0	8.0	0.9
5	A	20.4	5.6	4.0	2.3	2.2	0.9
	B	20.8	6.4	3.6	2.8	2.3	1.1
6	A	15.6	4.9	4.0	16.2	12.4	1.1
	B	15.1	5.0	4.7	18.3	12.0	1.0
	C ¹	15.1	6.6	5.0	4.8	1.5	—
7	A	10.7	8.4	7.4	7.2	4.8	1.0
	B	9.5	7.1	5.0	4.0	3.0	0.7

¹ Deeply anesthetized with Na-Amytal.

Individual graphs were plotted for each experiment and were seen to be separable into 4 general patterns. A typical representative of each of these patterns is plotted in figure 1, together with the numbers of experiments of which each curve is typical. *Experiment 15*, in which sodium amyral anesthetic was used in place of sodium pentothal, is not included in this group.

In the 3 experiments wherein a very high peak in O₂ consumption was noted the dogs exhibited violent shivering. The 2 dogs showing the moderate rise in metabolism likewise showed a moderate shivering response, while the 7 showing a very late, small rise shivered only minimally. It is of interest that, in the latter case, the shivering began approximately 25 minutes after pentothal anesthetization, which is in accord with the usual duration of this anesthetic in the absence of cold. But

seemingly at the temperature of emergence from the barbiturate depression the organism is not capable of great shivering.

It would appear from our data that the O_2 consumption response of the dog is largely an individual one inasmuch as 5 of the 7 dogs showed the same category of response both times they were cooled. The only pattern which was not repeated by at least 1 dog was that of curve B, figure 1, wherein both dogs which exhibited this response on the first cooling showed an altered pattern the second time, one falling into the pattern of curve A and the other of curve C.

The effect of deep narcosis on O_2 consumption during hypothermia was investigated by cooling for a third time the dog which twice under sodium pentothal showed the very high peak of curve A, figure 1. In this instance sodium amytal (50 mg/kg.) was the anesthetic and in this experiment (no. 15, table 1) no shivering was evidenced

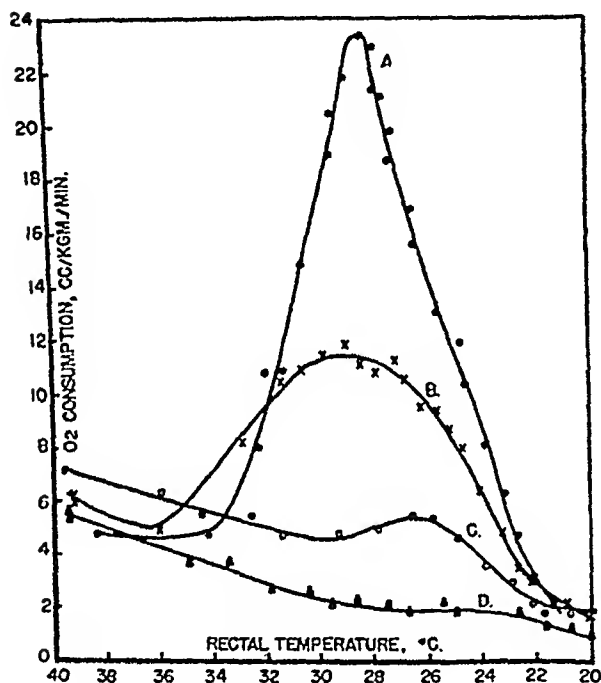


Fig. 1. TYPICAL EXAMPLES of the 4 types of oxygen consumption exhibited by hypothermic dogs. The no. of experiments in which each pattern was shown was as follows: A-3, B-2, C-7, D-2.

and the metabolism fell linearly to 24°C. rectal temperature, at which point respiration ceased. Resuscitation was instituted and successful rewarming effected. Three months later this animal was again cooled and rewarmed under pentothal anesthesia twice more with essentially the same response as experiments 6 and 13 of table 1.

Oxygen Consumption during Rewarming. In the first 7 experiments wherein rewarming was effected by spraying with warm water for about 5 minutes followed by exposure to room air of 25° to 28°C. shivering began between 24° and 28°C. rectal temperature. The O_2 consumption showed an almost simultaneous sharp rise and in all cases but one greatly exceeded the pre-immersion control level. The one exception occurred in the dog which did not shiver during cooling; and even in this case it is likely that, had the measurements not been terminated due to returning consciousness at 29.5°C., the control level would ultimately have been surpassed. Curve A of figure 2 is a typical O_2 consumption pattern of a dog rewarmed in air.

Fig. 2. OXYGEN CONSUMPTION PATTERN during rewarming in the hypothermic dog. Curve A: dog rewarmed in air ($25-28^{\circ}\text{C}.$) following 5 minutes of spraying with $40^{\circ}\text{C}.$ water; Curve B: same as A except that at rectal temperature $27.3^{\circ}\text{C}.$ dog was immersed in warm water ($40-42^{\circ}\text{C}.$) for duration of re-warming; Curve C: dog immersed in water at rectal temperature $20^{\circ}\text{C}.$ and removed to room air at rectal temperature $30^{\circ}\text{C}.$

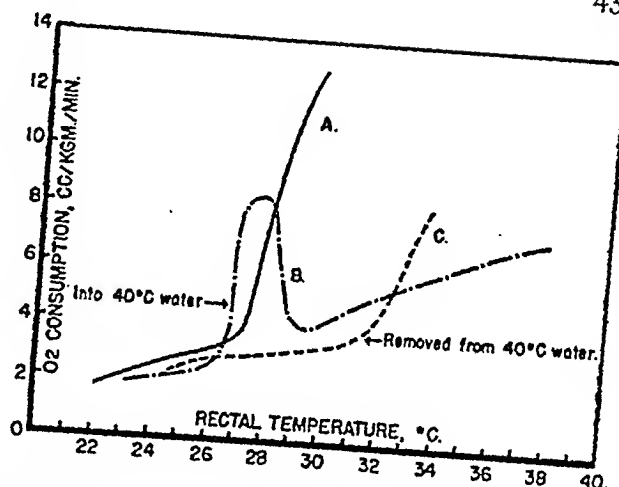


TABLE 2. RATE OF COOLING OF DOGS IMMERSED IN AN ICED WATER BATH ($2^{\circ}-5^{\circ}\text{C}.$)
Anesthetic: Pentothal Sodium

DOG	EXPER.	WT. kg.	TOTAL COOLING TIME min.	BEGINNING RECTAL TEMP. $^{\circ}\text{C}.$	LOWEST RECTAL TEMP. $^{\circ}\text{C}.$	MEAN COOLING RATE $^{\circ}\text{C}/\text{min}.$	TYPE OF O_2 CONSUMPTION PATTERN
1	A	9.5	68	38.8	21.2	0.259	Moderate rise
	B	7.9	59	38.9	20.0	0.320	Small, late rise
2	A	13.6	75	39.2	20.0	0.256	Small, late rise
	B	14.0	54	39.9	20.3	0.363	Small, late rise
3	A	15.6	52	38.5	19.4	0.367	Small, late rise
	B	14.0	67	40.0	21.8	0.272	Small, late rise
4	A	14.0	127	39.7	19.6	0.158	Moderate rise
	B	12.8	115	39.8	21.8	0.156	Very great rise
5	A	20.4	92	40.1	20.1	0.218	No rise
	B	20.8	80	39.5	21.2	0.229	No rise
6	A	15.6	164	39.7	20.0	0.120	Very great rise
	B	15.1	140	38.8	21.7	0.122	Very great rise
	C ¹	14.4	82	38.9	24.0	0.182	No rise
7	A	10.7	70	39.9	20.6	0.276	Small, late rise
	B	9.5	53	38.9	21.0	0.338	Small, late rise
168 ²		11.0	100	39.0	20.3	0.187	Dead
335 ²		9.4	101	40.0	20.0	0.198	Dead
336 ²		6.0	121	39.0	20.0	0.157	Dead

¹ Deeply anesthetized with Na-Amytal.

² Freshly killed dogs.

In the second half of the experiments it was found that the pronounced shivering and concurrent sharp O_2 consumption increase on rewarming could be controlled by immersion in a bath of warm water ($40^{\circ}-42^{\circ}\text{C}.$). The shivering, regardless of mag-

nitude, could be stopped almost immediately and the O_2 consumption would, within a short time, fall back to a line connecting the pre-immersion control level with that of $20^\circ C$. A typical representative of this pattern is shown in curve B of figure 2.

At no time during the course of these experiments was shivering observed while the dog was immersed in warm water. However, removal from warm water to air was invariably, and often within seconds, followed by the initiation of shivering. In one instance the animal was kept in warm water to a rectal temperature of $30^\circ C$. with no evidence of shivering. Shortly after removal shivering began and the O_2 consumption curve took a sharp upward turn. This response is plotted as curve C of figure 2.

Rates of Cooling. Through the use of the temperature recorder the opportunity was presented for the determination of precise rates of cooling. These data are presented in table 2, from which it may be seen that the mean cooling rates vary considerably between dogs as well as within the same dog on different days. Four of the 7 dogs showed a faster cooling rate on the second exposure while 1 showed a

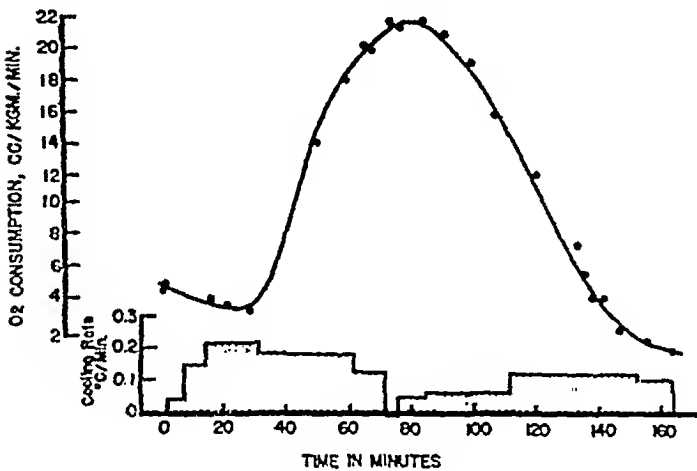


Fig. 3. CHANGES IN RATE OF cooling and O_2 consumption of a dog immersed in an iced bath. This dog showed great shivering.

slower rate and in 2 there was effectively no change. The fact that the mean cooling rate on the second exposure was 8 per cent faster than on the first is not thought to be significant.

If the mean cooling rates be separated in accordance with the 4 categories of O_2 consumption discussed previously a good correlation is obtained. In the 3 experiments wherein the O_2 consumption reached a high peak, the mean cooling rate was $0.133^\circ C/min.$; in the two in which the rise was moderate, $0.208^\circ C/min.$; in the 7 showing the late, slight rise, $0.313^\circ C/min.$; and in the case of the dog which showed a progressive declination both times, $0.223^\circ C/min.$ Only in the latter case is the value not in complete accord with the amount of heat production by the animal as evidenced by his O_2 consumption. In the 1 experiment wherein the dog that had shivered violently during both coolings under pentothal was cooled again under sodium amytal with no evidence of shivering the cooling rate increased 54 per cent.

Data from 3 previous experiments involving freshly killed dogs are added to table 2. In these dogs it is seen that the mean cooling rate to $20^\circ C$. does not differ materially from that of the deeply anesthetized dog and is slower than all but 4 of the 14 observations on lightly anesthetized dogs.

The rate of cooling at any instant is closely associated with the metabolic activity. Evidence for this is seen in figure 3 in which the cooling rate is plotted against the O_2 consumption in a dog which exhibited a great deal of shivering. Of primary interest in this graph is the fact that for a period of 4 minutes during the peak of the O_2 consumption and shivering, this dog was able to effect a reversal of the cooling trend and actually rewarm slightly even in the face of the water bath temperature of 3°C .

In the absence of shivering and metabolic increase during cooling the rate of cooling becomes high soon after immersion and progressively decreases as the rectal temperature of the animal approaches that of the environment. This relationship is pictured in figure 4.

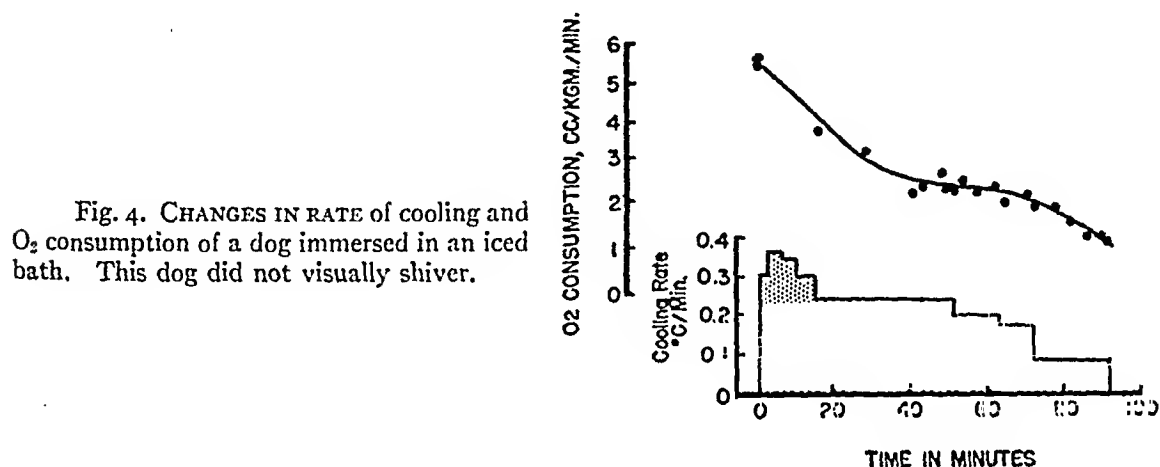


Fig. 4. CHANGES IN RATE OF COOLING AND O_2 consumption of a dog immersed in an iced bath. This dog did not visually shiver.

DISCUSSION

Although the use of anesthetized animals for observations of this type has been questioned from a physiological point of view (1) it proved desirable from many standpoints to induce a degree of light narcosis during the initial stages of cooling. Spealman (2) has shown that hypothermia in the unanesthetized dog is difficult to obtain by immersion in cold water. Cold itself, after sufficiently low body temperatures have been reached, acts as a narcotic which as pointed out by Haterius and Maison (3) is superimposed upon a long-acting anesthetic. For this reason pentothal sodium, which under normal conditions has a relatively short duration of action, was used on the theory that the cold would ultimately replace, rather than augment, the barbiturate effect. No direct proof for this supposition can now be offered, but this problem is being investigated.

The use of rectal temperature as a criterion of body temperature in immersion experiments has also been questioned (4). There can be no doubt that in this type of experiment temperatures of different organs will vary appreciably, especially at the lower ranges. However, if care is taken in positioning the rectal thermocouple its temperature does not differ widely from that of other deep areas of the body above a temperature of 22°C . A fuller discussion of these temperature relationships will be included elsewhere.

Grosse-Brockhoff and Schoedel (5) and Woodruff (6) have reported observations of O_2 consumption in the hypothermic dog. The former authors found a maximum

increase of 6 times, usually 3 to 4 times, in the early phase of the cooling of lightly anesthetized dogs. They reported the peak to be passed at 33° to 30°C ., a value somewhat higher than in our experiments. Likewise they reported that the metabolic level had always sunk below the pre-immersion value before the rectal temperature reached 25°C . In the 5 experiments of our series wherein the shivering was considerable the O_2 consumption did not sink below the pre-immersion control until a somewhat lower temperature was reached.

Neither of the above groups cooled their animals to a temperature as low as 20°C . nor did they report classes of response during cooling. The fact that only below about 23°C . rectal temperature, below which shivering is no longer a factor, is it possible to predict with any degree of accuracy the O_2 consumption of a given dog is believed to be an important aspect of our findings. It is entirely conceivable that this considerable difference in response of dogs is related to their inherent susceptibility to barbiturate anesthesia. It is not, however, correlated with quantity of pentothal. It is probable that, with enough animals, the 4 classes of response described above would give way to a complete spectrum of response varying from the very high peak in the dogs that shiver freely to the total absence of any peak in those dogs which fail to mobilize their defenses to the point of shivering. Moreover, in the absence of any depressant effects of anesthesia, it is likely that the predominant effect would be nearer the top of this spectrum than at the end wherein no increase was apparent.

Many authors (7-12) have reported an initial stimulation followed by depression in the metabolic level of unanesthetized rats subjected to cold air or cold water. The peak does not appear to be as high, relative to pre-cooling controls, as in the case of dogs. This is doubtless related to the fact that the shivering ability of the rat is generally less than that of the dog.

In the case of human patients, the observations have largely been made after prolonged exposures to cold. Smith and Fay (13) reported a BMR reduction of 6 per cent to 25 per cent, but Dill and Forbes (14) found a decrease in only 6 of their 28 observations made at temperatures of 25.5° to 38°C . Talbott (15) believes that the metabolism may well remain above the based level throughout the period of hypothermia, while Herrmann (16) and Geiger (17) hold that with prolonged hypothermia the BMR will be reduced, possibly showing a short period of stimulation early in the cooling.

The sudden and dramatic cessation of shivering upon immersion in warm water during the rewarming phase of these experiments was striking. The onset of shivering following removal from warm water to room air at rectal temperatures between 24° and 30°C . was almost as sudden. Uprus, Gaylor and Carmichael (18), using human subjects, observed a rapid shivering response to hot and cold baths, but, from their measurements of concurrent rectal temperatures, concluded the effect was of central origin and always correlated with a rise or fall of blood temperature. Jung, Doupe and Arnold (19) take exception to this view and hold that peripheral stimuli play a major rôle in this effect. Hemingway (20), using diathermy treatment on

dogs, found results which seem to substantiate Uprus *et al.*, but, from the suddenness of the response, our results favor the theory of peripheral origin.

Our data on rates of cooling do not appear to confirm a close relationship to body size. It is possible, however, that this effect was masked by the large variability in shivering. It is significant that in 10 of our observations the mean cooling rate was faster than is the case with a dead dog and only in 4 experiments, in which the shivering was great and prolonged, was the live animal able to effect a slower cooling rate. It is thus apparent that the dog is unable to effect sufficient peripheral vasoconstriction to prevent the conduction of a significant amount of heat to the surface even when exposed to extreme cold.

SUMMARY

Seven lightly anesthetized dogs were each cooled twice in an iced water bath to a rectal temperature of 20°C. and rewarmed in room air at 25° to 28°C. and/or warm water at 40° to 42°C. The O₂ consumption and rates of temperature change were recorded continuously. One of the dogs which shivered considerably on both coolings was subjected to a third cooling under deep, prolonged anesthesia. In addition, 3 observations of cooling rates of dead dogs are presented.

It is shown that the O₂ consumption varied directly with the shivering response and in the 14 experiments 4 distinct patterns were detectable. It is suggested that variations in shivering response patterns may be a function of susceptibility to barbiturate anesthesia.

Below 23°C. rectal temperature shivering is no longer a factor and the O₂ consumption of all dogs falls to approximately one third that of the pre-cooling control level.

During rewarming all dogs shivered greatly when exposed to room air, beginning between 24° and 28°C. rectal temperature. The shivering could be stopped almost immediately by immersion in warm water. The O₂ consumption pattern followed very closely that of the shivering.

In 4 of the experiments, through profound shivering the dog was able to delay the body cooling such as to make the mean cooling rate slower than that of a dead animal. In the other 10 experiments the cooling rates were all faster than for dead dogs.

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MAN'S RESPIRATORY RESPONSE DURING AND AFTER ACCLIMATIZATION TO HIGH ALTITUDE¹

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INCREASED pulmonary ventilation in response to lowered oxygen pressures has long been recognized in man. For a given oxygen pressure, however, this response is not the same for acclimatized and unacclimatized man. In order to analyze the changes which occur during the process of adaptation, various types of experiments have been designed. It is our objective 1) to describe the difference in alveolar air composition between persons acclimatized to various altitudes and persons residing near sea-level who are acutely exposed to similar altitudes and to predict on this basis the ventilatory acclimatization for any altitude, 2) to verify this prediction in experiments on 3 subjects acclimatized to an altitude of 9500 feet for a period of 3 weeks, 3) to describe the respiratory response of men acutely exposed to lower and higher oxygen pressures *after* acclimatization to various altitudes, and 4) to present data which indicate a greater sensitivity of the respiratory system to CO₂ after acclimatization to altitude.

Difference between Alveolar Air Composition and Ventilation of Acclimatized and Unacclimatized Man at Similar Oxygen Pressures. The alveolar gas composition of permanent residents as well as sojourners acclimatized to altitude have been collected from reports of various mountain expeditions and are presented in table 1. The alveolar respiratory quotient has been computed in each case from the alveolar gas equation (1, 2).

$$R. Q. = \frac{.791 \text{ pCO}_2}{.209 (B - 47 - \text{pCO}_2) - \text{pO}_2} \quad \text{Equation 1}$$

The values obtained by Fitzgerald (24) are not indicated in the table since they cover various altitudes from 4000 to 14,000 ft. on permanent residents of Colorado, U. S. A. The averages of her determinations, however, are incorporated in figure 1 and since only CO₂ determination was made, the alveolar oxygen pressure was computed by the alveolar gas equation, assuming a resting R.Q. of .83.

As far as can be ascertained, all samples were collected during rest, and from a forced expiration following a normal expiration. The samples of Helmholtz and Boothby (3), Boothby (2) and Hall and Wilson (4) were taken at the end of inspiration and have been corrected in figure 1 and table 1 to 'end-expiration' samples by adding

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1 mm. CO₂ and subtracting 2 mm. O₂ (5). It must further be appreciated that posture changes the resting alveolar gas concentrations considerably. It had to be assumed that all observations in the literature were made in the sitting posture.

TABLE 1. ALVEOLAR AIR COMPOSITION OF SOJOURNERS AND PERMANENT RESIDENTS AT VARIOUS ALTITUDES AS RECORDED IN THE LITERATURE

NO.	ALTITUDE 1000 FT.	BAR. PRES., MM. HG	NO. SUBJECTS	ALVEOLAR			AUTHORITY
				pCO ₂	pO ₂	R.Q.	
1 ²	0	760 ¹	16	38.0	106.0	.858	(3)
2	.55	748	22	38.1	100.7	.797	Our data
3 ²	1.00	733	35	37.7	100.3	.853	(2)
4	4-14		132				(24)
5 ²	6.2	610	32	33.3	79.5	.866	(4)
6	9.2	543	10	34.0	64.0	.828	(25)
7	10.0	525	8	31.5	61.9	.813	(27)
			3	31.5	63.9	.845	
6	12.5	489	10	30.0	58.0	.850	(25)
7	14.0	446 ¹	3	25.9	51.6	.776	(27)
8	14.2	458		26.8	53.7	.793	(26)
9	14.2	458	4	27.9	52.6	.807	(18)
10	15.4	429	11	28.0	46.9	.820	(28)
	17.5	401	10	25.6	42.3	.761	
	20.1	356	9	21.4	37.7	.761	
11	21.2	331 ¹	2	17.7	42.5	?	(29)
	22.8	310 ¹	2	15.6	37.0	.845	
12	22.7	337	1	17.7	40.7	.860	(30)

¹ Based on Altitude-Pressure Tables, U. S. Stand. Atmos. ² Alveolar values corrected for end-expiratory samples as explained in text.

TABLE 2. AVERAGE ALVEOLAR AIR COMPOSITION OF 22 MEN EXPOSED TO VARIOUS SIMULATED ALTITUDES IN A LOW-PRESSURE CHAMBER. SAMPLES WERE TAKEN EVERY 10 MIN. DURING AN EXPOSURE WHICH LASTED ON THE AVERAGE ONE HOUR

ALTITUDE 1000 FT.	BAR. PRES. MM. HG.	NUMBER OF DETERMIN.	ALVEOLAR					Alv. Vent. Ratio (calculated)
			pCO ₂	S.D.	pO ₂	S.D.	R.Q.	
0.55	748	130	38.1	3.5	100.7	6.5	.797	1.00
12.0	483	117	37.4	3.0	47.6	4.7	.830	1.06
16.0	412	48	31.7	4.7	40.7	6.1	.869	1.31
18.0	379	160	30.3	3.8	36.5	4.5	.905	1.43
20.0	349	36	26.9	4.7	34.3	5.1	.918	1.62
22.0	321	23	24.6	3.7	31.3	4.6	.937	1.81

The acute exposures to low oxygen pressures have been carried out by means of a low pressure chamber. Twenty-two young men were exposed, on the average, 1 hour at various simulated altitudes. By far the greatest part of the data was obtained on 8 men who served as regular subjects in these experiments lasting over 3

months. All of these men participated daily either as observers or subjects and were thus well trained and had complete confidence in their work. This absence of all anxiety is a prerequisite in obtaining reliable and typical respiration responses. Alveolar samples were collected approximately every 10 minutes in the sitting position from a forced end-expiration and analyzed by the Haldane method or by the automatic oxygen and carbon-dioxide analyzers described by Rahn *et al.* (5). The alveolar gas concentrations for the acute exposure to various altitudes are listed in table 2 and represent the average for the whole period of exposure.

When the alveolar oxygen and carbon dioxide tensions are plotted on the Fenn $\text{CO}_2\text{-O}_2$ diagram we obtain 2 distinct curves differentiating acclimatized from unacclimatized men (fig. 1). The iso-altitude line on such a diagram is represented by a

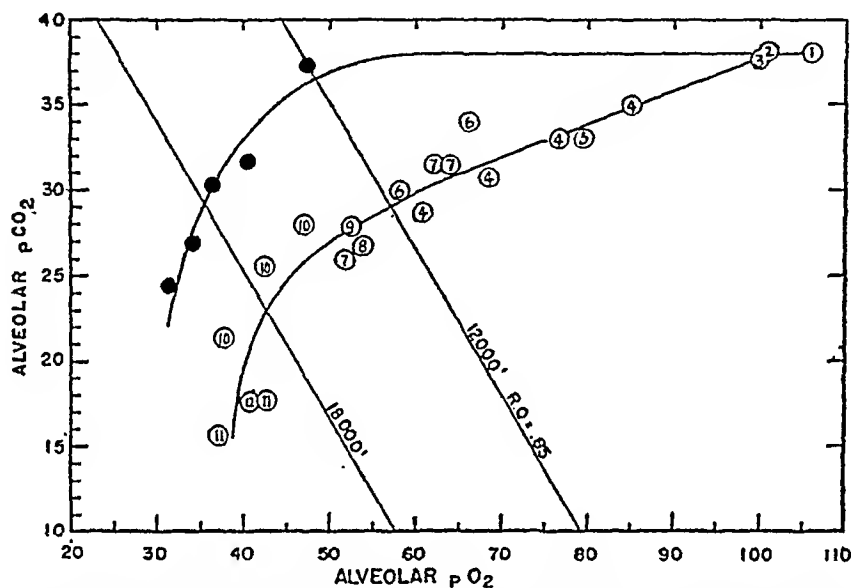


FIG. 1. DIFFERENCE IN ALVEOLAR AIR COMPOSITION between man acutely exposed to various altitudes (solid dots from table 2) and man acclimatized at various altitudes (open circles; the numbers refer to references in table 1). Two iso-altitude lines for a R.Q. = .85 are represented as diagonals.

diagonal the origin of which lies at the inspired oxygen tension for each altitude and the slope of which is determined by the respiratory quotient (r). As an example, the figure includes the iso-altitude lines for 12,000 and 18,000 feet at a respiratory quotient of .85. Any alveolar air or expired air values at this R.Q. must lie on these lines at these altitudes. Under similar conditions altitude diagonals for other pressures lie parallel to those indicated in figure 1.

Two striking facts are revealed: 1) at a given altitude the alveolar $p\text{CO}_2$ is always lower and the O_2 higher in the acclimatized man; 2) the alveolar $p\text{CO}_2$ does not fall in the unacclimatized man until an alveolar $p\text{O}_2$ of 50-60 mm. Hg is reached, whereas in the altitude residents the fall begins at approximately 100 mm. Hg, or sea level.

From the alveolar gas concentration the relative alveolar ventilation can be computed from the equation of Fenn (1),

$$V_a = \frac{.864 X_0 Q}{\text{alv. } p\text{CO}_2}$$

Equation 2

where V_a equals alveolar ventilation in liters per minute B.T.P.S.; X_o equals the oxygen consumption in ml. S.T.P.D.; Q , the respiratory quotient, and .864 is a constant. This formula is valid when the inspired air contains no CO_2 .

In order to compute the relative alveolar ventilation the assumption has to be made that the resting oxygen consumption is unaffected by the altitude. The value obtained at the common origin of these 2 curves in figure 1 at the alveolar $\text{pCO}_2 = 38.0$ is designated as the *alveolar ventilation ratio* of 1.00. The values taken for calculating the alveolar ventilation curve for the acclimatized men are as follows: the CO_2 is based upon the average curve presented in figure 1, while for the R.Q. the value of .82 was used which represents the average R.Q. for all determinations in table 1.

For the acute exposure the ventilation ratio was computed only from the alveolar values obtained during the last 10 to 20 minutes of exposure where the R.Q. had returned to an average value of .85 (6). This makes the R.Q., for our purposes, practically identical with those obtained for the acclimatized people. Thus, for both groups the alveolar ventilation becomes only a function of the pCO_2 and in figure 2 the alveolar ventilation ratio, $V_a R.$, as well as the alveolar pCO_2 can be plotted as ordinates against the alveolar pO_2 and the altitude lines.

Furthermore, the change in serum-bicarbonate of the blood to be expected after acclimatization is completed, can be indicated on the same diagram. If one assumes that the pH upon acclimatization returns to normal (7-10), it follows from the Henderson-Hasselbalch equation that the bicarbonate level likewise becomes a function of the alveolar pCO_2 only.

Figure 2 is particularly helpful in predicting the pathway of the alveolar air composition and alveolar ventilation during the process of acclimatization for any altitude. If we accept these 2 curves, then the process of this adaptation must proceed along a line connecting them. The exact acclimatization pathway, however, is already definitely prescribed by the iso-altitude diagonal as long as the R.Q. remains within normal limits. Thus, for example, the pathways for 5, 10, 15 and 20 thousand feet altitude for an R.Q. of .85 are indicated in figure 2. The acute exposure value after 30 to 60 minutes at these altitudes will lie at the intersection with the unacclimatized curve. The acclimatization pathway will in each case proceed down the altitude diagonal and come to rest at the final intersection with the acclimatized curve. The rate at which this process takes place has been established for the 10,000-ft. altitude and is discussed below. It is of interest to compare the alveolar values obtained by Houston and Riley (8) on 4 men exposed gradually over 4 weeks to increasing altitudes up to 22,000 ft. in a low-pressure chamber. These men never completely acclimatized to any altitude and their alveolar values, described recently in greater detail (38), fall approximately half-way between the 2 curves in figure 2.

The alveolar ventilation curve for acute exposure is of great interest since it indicates no increase in ventilation until the alveolar pO_2 drops to values below 60 mm. Hg. This corresponds to an altitude of approximately 12,000 ft. or to breathing a mixture of 13 per cent oxygen in nitrogen at sea level and is in general agreement with the recent data of Soley and Shock (11), Dripps and Comroe (12) and Rahn and Otis (6) who measured the *total ventilation* at various oxygen pressures. Furthermore, the alveolar ventilation ratios calculated from the extensive alveolar CO_2 and O_2

determinations of Boothby (2) yield values very similar to our own. Thus, it seems quite certain now on the basis of direct measurements as well as calculations based upon the alveolar gas composition that ventilation in man acutely exposed to low oxygen pressures does not increase measurably until the alveolar oxygen pressure drops to about one half of normal (50-60 mm. Hg).

On the other hand, the alveolar air composition of permanent residents or recently acclimatized men indicates a greater than normal (sea level) ventilation. Thus, the hypoxic stimulus is inhibited during at least the first hour in the acutely exposed unacclimatized man. Since below 12,000 ft. altitude this inhibition cannot be due to hyperventilation alkalinity, the only factor that suggests itself is the alka-

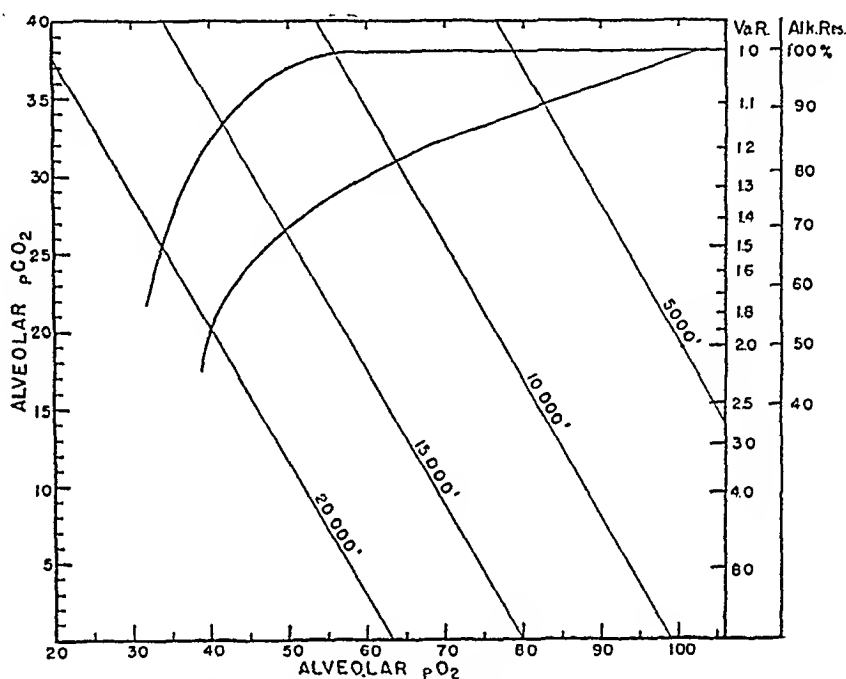


FIG. 2. DIFFERENCE IN ALVEOLAR AIR COMPOSITION and relative alveolar ventilation ratio, V_A/R , between unacclimatized man (upper curve) and acclimatized man (lower curve). The calculated change in the alkali reserve, Alk. Res., does not apply to unacclimatized man and applies to the acclimatized man only on the assumption that the arterial pH has returned to normal. The iso-altitude lines are constructed for an $R.Q. = .85$.

linity produced by the lowered saturation of the arterial blood. At 12,000 ft. this amounts to about 0.008 pH unit at a pO_2 (alveolar) of 50 mm. Hg and pCO_2 or 38 mm. Hg or an HbO_2 per cent of 86. On the basis of Gray's multiple factor theory this change in pH would produce an inhibition of about 16 per cent of the alveolar ventilation which is just about balanced by a 20 per cent ventilation increase stimulus shown in the acclimatized man at this altitude (13).

In order to increase the ventilation, therefore, at this altitude one may speculate that compensatory changes in the blood pH must occur bringing the pH back to normality before the hypoxic stimulus can effectively exert itself. This is not attained at the end of one hour exposure but appears soon thereafter as will be discussed below.

Acclimatization of the Respiratory Responses Tested at 9500 Ft. During the sum-

mer of 1946 three of us took up residence at the University of Wyoming Science Camp located in the Snowy Range at an altitude of 9500 ft. ($B = 535$). Prior to this residence daily determinations were made at Rochester, N. Y., to establish the normal alveolar gas concentration, ventilation volume, respiratory rate and breathholding performance tests. Instrumentation was similar to that used in previous experiments (5). All tests were run between 10 A.M. and 12 M. The subject was comfortably

TABLE 3. ACCLIMATIZATION OF SUBJECTS, A.O. AND R.S. AT 9500 FT. ALTITUDE

DATE	n	ALVEOLAR			OXIMETER HbO ₂ %	VENTILATION L/MIN RTPS	BREATHS/MIN	BREATH HOLDING BREAKING POINT					EXPOS. TIME	
		pCO ₂	pO ₂	R.Q.				pCO ₂	pO ₂	R.Q.	HbO ₂ %	Time held, seconds		
7-1	744	37.7	101.6	.83		8.58	12.3	49.0	52.9			48		Rochester, N. Y.
7-3	754	38.2	103.6	.84		8.78	11.6	50.1	50.2			51		
7-4	755	38.2	104.6	.85		9.08	11.7	48.9	50.4			53		
7-5	753	38.4	104.2	.86		9.24	13.2	51.0	53.6			54		
7-6	749	37.7	103.2	.85		8.76	13.1							
7-3	533	38.9	57.2	.85	89	7.91	12.6	47.4	36.0		71	34	1 hr.	
7-10	535	34.0	59.9	.77	88	7.45	11.1	41.8	36.0	0.58	68	33	12 hr.	Wyoming—9500 ft.
7-10	536	33.5	59.9	.82		8.63	12.7						18 "	
7-11	536	33.1	63.7	.84	91	9.20	12.3	41.8	40.6	0.64	74	29	36 "	
7-12	536	32.5	61.2	.77		8.48	12.7	40.3	36.7	0.57		32	60 "	
7-14	536	31.7	62.9	.78	91	8.55	12.9	39.3	39.6	0.59	74	34	84 "	
7-15	536	30.6	62.3	.74	91	8.67	13.3	38.8	40.6	0.59	75	35		
7-16	535	30.6	63.9	.77	90	9.10	13.0	39.0	42.2	0.61	75	30		
7-18	535	31.0	63.3	.77	90	8.03	12.7	38.4	43.0	0.60	77	28		
7-21	538	30.7	63.8	.75		8.65	12.5	38.4	42.5	0.59		29		
7-22	535	31.8	62.9	.76	89	9.35	12.8							
7-28	538	31.9	63.8	.80	88	8.96	10.9	40.0	40.0	0.58		30		

Averages on three subjects A.O., R.S. and H.R. before and after acclimatization

Before	751	38.0	103.2	.84		8.68	11.5	49.7	54.7	0.48		58	Roch.	Wyoming
After	536	30.9	63.3	.76	90	8.51	11.5	37.9	44.7	0.61	77	29	5th to last day	

seated and the alveolar air continuously analyzed. When after 10 minutes or longer the alveolar gas concentrations had stabilized, the values were recorded every minute for 5 minutes and averaged. During this period the ventilation volume and breathing rate were automatically recorded on paper tape and averaged. This procedure was followed by 2 breathholding tests (see below). Two days prior to leaving for the mountains similar tests were made in our high-altitude chamber at the end of 1 hour exposure to an altitude of 9500 ft. This acute exposure as can be seen in table 3 showed no change in the alveolar pCO₂ or ventilation as had been predicted.

Twelve hours after arrival at the altitude camp the test was repeated until the end of the 3-week stay. These data are based largely upon 2 subjects, since the third had to set up equipment in advance and was partially acclimatized at the time the tests could be started.

Table 3 indicates that the CO_2 had already dropped nearly halfway to its final value at the end of a 12-hour exposure and plateaued off at the end of 4 days at this altitude where it remained constant. This progression is shown on the O_2 - CO_2 diagram in figure 3 and follows the predicted course except for the low R.Q. values. Not only was the pathway as predicted during the process of respiratory acclimatization, but also the final values after acclimatization fell exactly upon the predicted

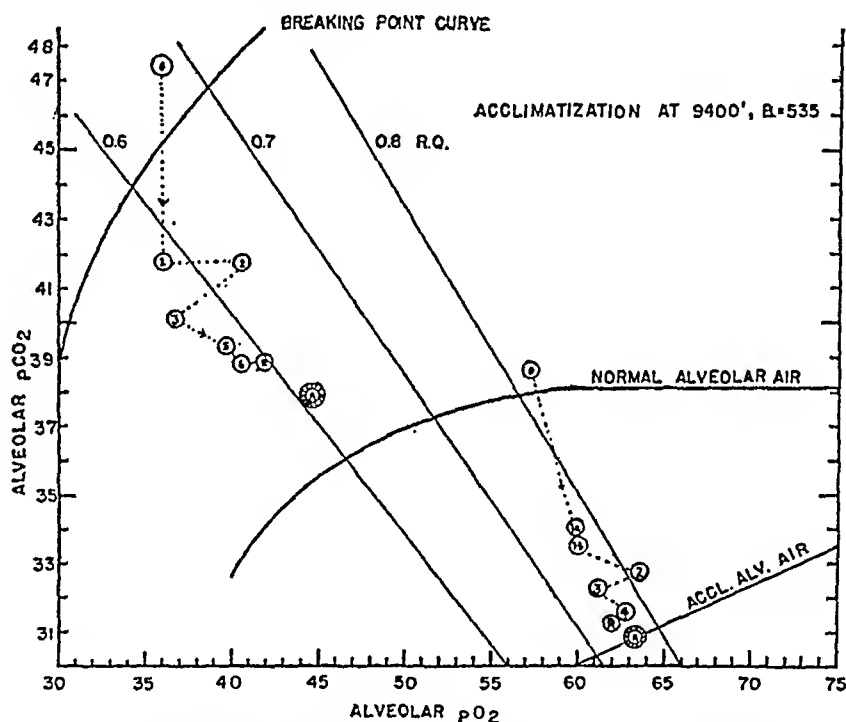


FIG. 3. NORMAL AND BREATH HOLDING BREAKING POINT alveolar air composition (dotted line) of 2 subjects during acclimatization at 9500 ft. The numbers in the circles indicate the days of residence at this altitude. The number o indicates 1 hour exposure and R the 5-18th day. 'A' indicates the average for all 3 subjects after respiratory acclimatization was complete. The 2 alveolar air curves (solid lines) are based on figure 2, while the breaking point curve is based on previous data (19) shown also in figure 7.

CO_2 level. Precisely the same change was recently observed by Hetherington, Luft and Ivy (14) when they reported an average difference of 7 mm pCO_2 after acclimatization to 10,200 ft.

The fall of the alveolar R.Q. from an average of .84 to .76 (table 3) is of great interest. Although we have no satisfactory explanation, it should be pointed out that Hasselbalch and Lindhard (15) made similar observations during their 17-day stay at an altitude of 10,800 ft. Very recently the studies of Hetherington, Luft and Ivy (14) have confirmed these observations on 27 men who were taken from an elevation of 750 ft. to a 10,200-ft. level for a period of 2 weeks. The alveolar R.Q. was altered from an average of .80 to .75. Upon return to near sea level they observed a very slow return to the original value.

On the basis of the change in the alveolar $p\text{CO}_2$ and R.Q. before and after acclimatization (last column, table 3), the predicted increase in alveolar ventilation should have been $38/31 \times .76/.84$ or 11 per cent above the Rochester value provided that the resting oxygen consumption remained unaltered. Actually no significant change was observed. This could most easily be explained by a lower resting oxygen consumption at altitude. However, the studies and review of the literature by Lewis, Illif and Duval (16) indicate no change in basal metabolism in a comparison of 15 independent studies ranging from sea level to 7000 ft. In our subjects the number of breaths per minute was not altered, nor was there any appreciable change in the blood saturation as measured by the Millikan oximeter from that obtained during acute exposure at Rochester.

Averages for all 3 subjects at Rochester and after completion of respiratory acclimatization at Wyoming are given at the bottom of table 3. The rate of pulmonary

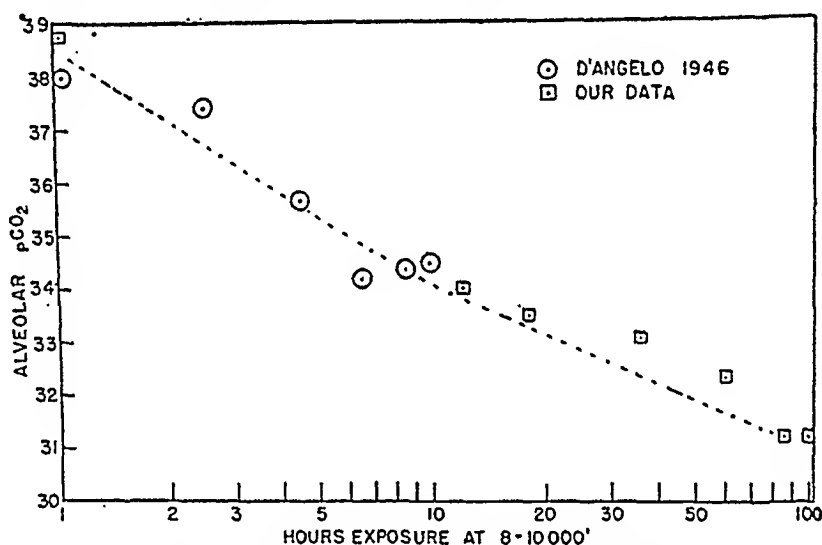


FIG. 4. RATE OF CHANGE of the alveolar $p\text{CO}_2$ during acclimatization to an altitude of 8000-10,000 ft.

adaptation is best visualized in figure 4, in which the alveolar CO_2 level is used as the index of acclimatization. This is completed by the end of 90 hours at this particular altitude. The rate must be governed by the degree of alkalinity incurred from the hyperventilation and the rate of base excretion from the kidneys. Our studies which start after 12 hours are well supplemented by those of D'Angelo (17) who exposed a large number of men in a high-altitude chamber to altitudes of 8 and 10 thousand feet for 10 hours. Although the alveolar gas was not determined directly in his studies, the CO_2 can readily be calculated from the ventilation equation, since all the pertinent data were given, if one assumes a constant dead space. Thus, a fairly complete picture of the rate of acclimatization is obtained for the first time, since other expeditions were always forced to spend several days at various intermediate altitudes and thus became partially acclimatized before reaching their final destination.

Respiratory Response of Man Acutely Exposed to Lower and Higher Oxygen Pressure after Acclimatization to Various Altitudes. The only extensive data are those of

Hall and Wilson (4) who acclimatized 32 subjects to an altitude of 6200 ft. at Colorado Springs. These subjects were then exposed to various altitudes in a low-pressure chamber and their alveolar gases analyzed. Our data extend these observations to 9500 ft. where we breathed a mixture of 12 per cent oxygen in nitrogen after acclimatization (table 4).

The breathing of high oxygen mixtures after acclimatization causes no immediate change in the alveolar $p\text{CO}_2$ and consequently no decrease in ventilation (table 4). This is in agreement with the recent work of Houston and Riley (8) who found that their subjects after being acclimatized to 22,000 ft. maintained their high ventilation and low CO_2 value after being brought back suddenly to the relatively high-oxygen pressure of sea level. It would seem that re-acclimatization to sea level must occur slowly by retention of base in a similar process, but in reverse to that described above,

TABLE 4. RESPIRATORY RESPONSE TO 100% AND 12% OXYGEN AFTER ACCLIMATIZATION TO 9500 FT. ALTITUDE

INSPIRED GAS AT 9500 FT.	SUBJECT	NORMAL ALVEOLAR RESPONSE			BREATH HOLDING BREAKING POINT			
		$p\text{CO}_2$	$p\text{O}_2$	Oximeter	$p\text{CO}_2$	$p\text{O}_2$	Oximeter	Seconds
100% oxygen	A. O.	31.7		100	46.9	442 ¹	100	90
	R. S.			100	51.2	437 ¹	100	74
	H. R.	32.9		100	53.5	436 ¹	100	173
	Average	32.3		100	50.3	438	100	112
11.77% oxygen in nitrogen	A. O.	25.2	32.8	68	30.3	23.8	58	19
	R. S.	22.6	37.9	78	27.7	28.3	64	25
	H. R.			74	29.4	30.6	67	14
	Average	23.9	35.3	73	29.1	27.6	63	19

¹ $p\text{O}_2$ values obtained by subtraction.

as demonstrated many years ago by Douglas *et al.* (18) upon their descent from Pike's Peak.

Figure 5 attempts to summarize the alveolar response of man exposed to various oxygen tensions after acclimatization. The solid line represents the alveolar values of the acclimatized individual as seen in figures 1 and 2. Point *A* is the sea level value and the dotted curve originating at this point is the acute response to low and high oxygen pressures. Point *B* is the alveolar value for man living at Colorado Springs at 6200 ft. and the dotted line again indicates the alveolar response when acutely exposed to lower oxygen pressures (4). Point *C* is the acclimatized point for approximately 10,000 ft., while the dotted line through this point indicates the response to lower as well as higher oxygen pressures. Similar curves could be drawn for other altitudes and the approximate alveolar response predicated as well as the ensuing acclimatization pathway. Thus as an example, if a man living at Colorado Springs were suddenly placed at an altitude of 18,000 ft. his alveolar pathway would start at *B*, jump to the intersection of the 18,000 ft. diagonal with the alveolar curve (dotted line) originating at *B*. From there it would travel down the 18,000 ft. altitude diag-

onal to its intersection with the acclimatized curve (solid line). On the other hand, if returned from 6200 ft. to sea level his alveolar pathway would travel from *B* on the dotted line to the right until it intersects the sea level diagonal and from there proceed slowly upward to point *A*.

Increased Sensitivity of the Respiratory System to CO₂ after Acclimatization. *A) CO₂ inhalation.* The first experiment measured the ventilation response to CO₂ added to the inspired air. The percentage composition of the various gases inhaled are listed in table 5 and give approximately the same CO₂ tension in the moist inspired

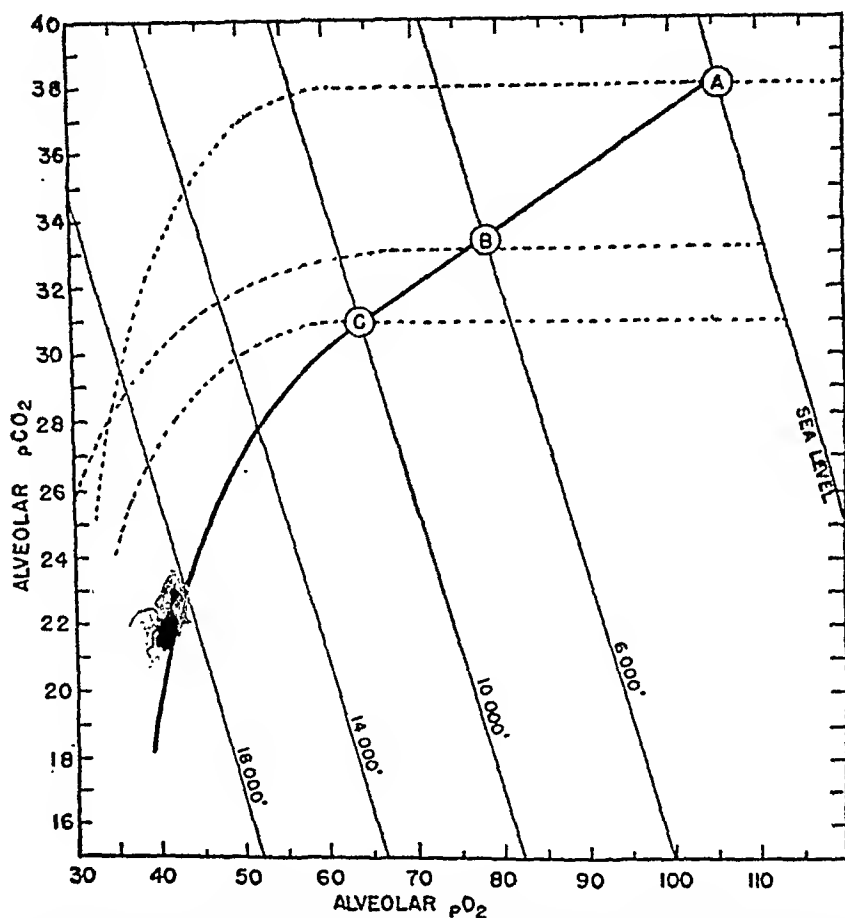


FIG. 5. ALVEOLAR AIR COMPOSITION of the acclimatized man (solid line) and the changes which occur (dotted line) when man acclimatized at sea level, (A); 6000 ft. altitude (B); and 10,000 altitude (C), is acutely exposed to lower or higher oxygen tension. See text.

air at both altitudes. The procedure was such that the subject inhaled consecutively air, low CO₂ and high CO₂ mixture each for 15 minutes from a demand regulator on each gas cylinder. Thus the change could be made instantaneously from one to the other without interruption. Minute to minute recordings were made of the alveolar gas composition, ventilation volume and rate, and ear oximeter. The last 5 minutes of each 15-minute exposure period were averaged. The 3 subjects were run on 2 occasions at both altitudes. The combined averages of all 6 runs are given in table 5.

Although the steady state was not attained with CO₂ breathing, as indicated by the low R.Q., the exposure time in all cases was the same and allows a comparison at

these 2 altitudes. Figure 6 indicates a greater ventilatory response for any given inspired CO_2 tension after acclimatization to 9500 ft. The ventilation increment is approximately 50 per cent greater over the range tested. Although breathing CO_2 increased the alveolar O_2 tension considerably it is expected that this in itself would not influence the response of the respiratory system during an acute exposure (see discussion above). If it did, the higher O_2 tension at altitude would tend to decrease the differences.

The sensitivity of the respiratory system might also be expressed in terms of change in alveolar pCO_2 which will produce a certain percentage increase in ventilation. If this is done for the high CO_2 mixtures at Rochester and Wyoming, we find that after acclimatization to altitude 0.75 pCO_2 induces the same ventilation increase as 1.00 pCO_2 at Rochester.

TABLE 5. EFFECTS OF CO_2 ADDITION TO THE INSPIRED AIR AT ROCHESTER, N. Y. AND AFTER ACCLIMATIZATION TO ALTITUDE. EACH FIGURE REPRESENTS AVERAGES OF TWO RUNS ON EACH OF THREE SUBJECTS (SEE TEXT).

INSPIRED GAS MIXTURE	INSPIRED pCO ₂	ALVEOLAR			OXIMETER	BREATHS PER MIN.	VENTIL. L/MIN. BTFS	VENTIL. RATIO
		pCO ₂	pO ₂	R.Q.				
Rochester (B=752 mm. Hg)								
air	0	37.9	104	.86		11.7	8.59	1.00
3.2% CO ₂	22.5	40.2	135	.75		12.9	14.58	1.70
22.3% O ₂								
5.2% CO ₂	36.6	47.1	126	.75		12.7	23.92	2.78
19.7% O ₂								
Wyoming (B=537 mm. Hg)								
air	0	30.4	65	.77	88	11.1	8.77	1.02
4.5% CO ₂	22.0	36.4	83	.71	92	12.0	16.98	1.98
20.6% O ₂								
7.0% CO ₂	34.2	41.5	91	.73	95	16.4	33.27	3.88
20.5% O ₂								

B) Breath-holding tests. The breath-holding tests were similar to those described by Otis, Rahn and Fenn (19). At the end of normal expiration the breath was held until the breaking point was reached and the alveolar air analyzed. The breath holding time and the ear oximeter reading were recorded. These data are summarized in table 3, while table 4 gives additional data for similar tests carried out breathing 12 per cent oxygen and 100 per cent oxygen after acclimatization was completed at 9500 ft. During the process of acclimatization the composition of the alveolar gas at the breaking point changes in much the same way as the alveolar gas before breath holding. This is shown by the 2 'pathways' plotted on the pO_2 - pCO_2 chart in figure 3.

On the basis of the various data in table 3 and 4 the breaking point curve and the normal alveolar curve after acclimatization have been drawn in figure 7 together with similar curves obtained previously on unacclimatized men (19). The vertical

distance between the alveolar air curve and the breaking point curve represents the magnitude of the CO_2 stimulus necessary to achieve the breaking point for a given oxygen tension. Thus this distance may be regarded as measure of the sensitivity of the respiratory center to CO_2 .

One can readily see that the vertical distance between these 2 curves is much greater at Rochester than after acclimatization to 9500 ft. ($B = 535$). At the right hand side of the graph CO_2 is the only factor concerned in achieving the breaking point, oxygen having no effect. Thus near sea level it takes a difference of about (65-38) 27 mm. Hg CO_2 to reach the breaking point while after acclimatization to 9500 ft. at the identical oxygen pressure a difference of 18 mm. Hg (50-32) is sufficient. If we regard the breaking point as an expression of ventilation response (the same at both altitudes) then it is obtained by a change of $\frac{1}{2}\frac{8}{7}$ or .67 pCO_2 at 9500 ft. as compared with $\frac{2}{2}\frac{7}{7}$ or 1.00 pCO_2 near sea level. This figure agrees reasonably well with the relative stimulus of .75 obtained with CO_2 inhalation (test described above).

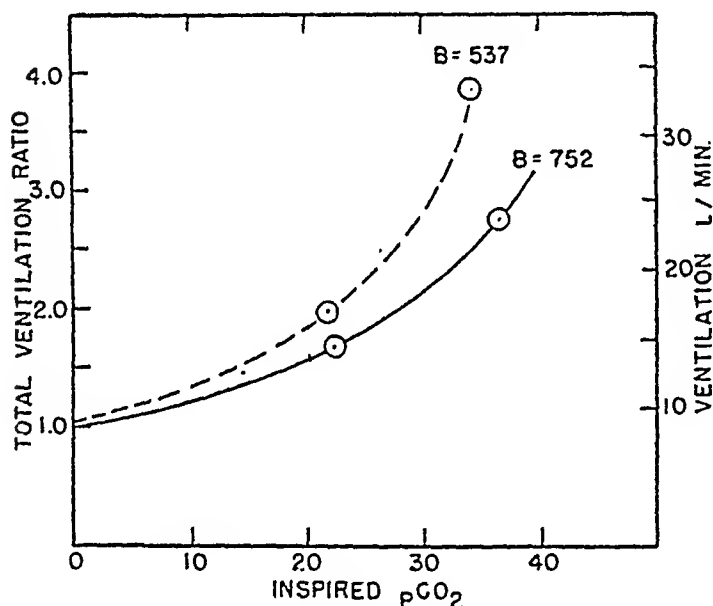


FIG. 6. EFFECT OF THE INSPIRED CO_2 TENSION UPON THE TOTAL VENTILATION AT SEA LEVEL AND AFTER ACCLIMATIZATION TO ALTITUDE. For details consult table 5.

From the CO_2 dissociation curve one may estimate the CO_2 content of the oxygenated plasma at a pCO_2 of 38 mm. Hg and a pH of 7.4 to be 55 vol. per cent. If we assume that after acclimatization to 9500 ft. the pH returns to normal, then the CO_2 content is reduced to 44 vol. per cent at a pH of 7.4 and a pCO_2 of 31 (breathing pure O_2). Under these conditions the computed pH at the breaking point for both altitudes is the same, namely 7.25. Thus under these conditions of breath holding with pure oxygen where oxygen lack does not enter as a stimulus it is very tempting to regard pH as the determining factor at the breaking point. Furthermore, the increased sensitivity of the respiratory system to CO_2 after acclimatization might merely be the effect of this gas on the reduced buffering capacity of blood and tissues. This of course would also explain the continuation of hyperventilation when man acclimatized to altitude is suddenly exposed to higher than normal oxygen pressures.

C) *Breath-holding time.* From the above discussion it should follow that when a man acclimatized to various altitudes breathes pure oxygen and then holds his

breath, the breath-holding time is simply a function of his alkali reserve. Thus, from figure 2 one can estimate an alkali reserve reduction to about 80 per cent of normal at 10,000 ft. while the breath-holding time on pure oxygen in 2 of our subjects (*R.S.* and *H.R.*) was reduced to 70 per cent. Unfortunately, no further data are available.

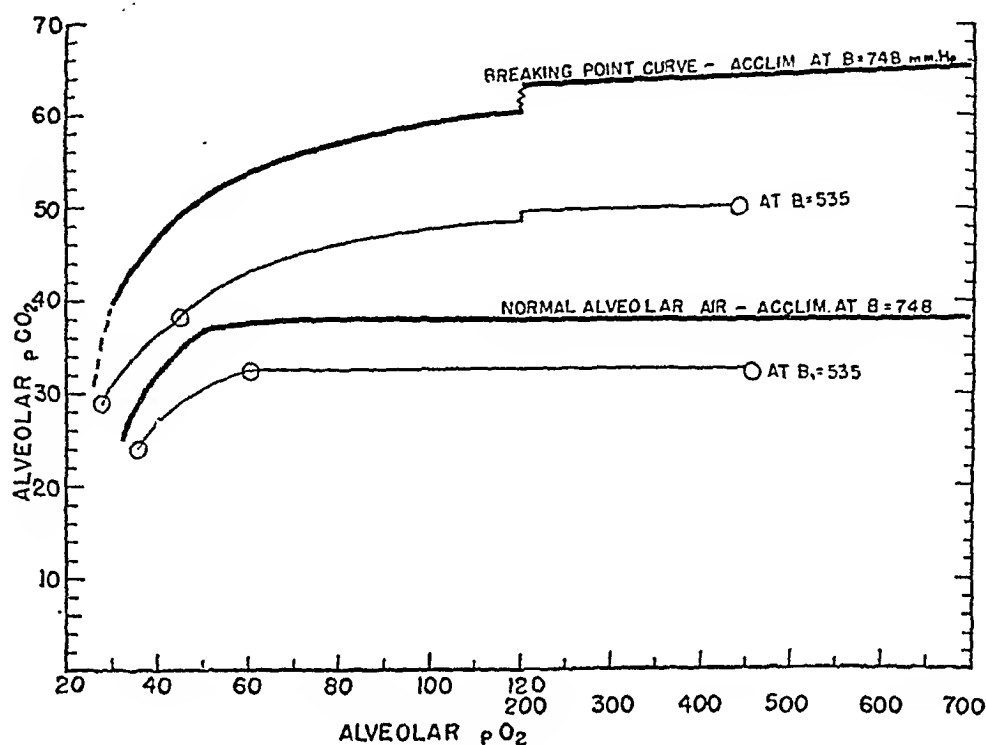


FIG. 7. NORMAL ALVEOLAR AIR COMPOSITION and that obtained after breath holding when man is exposed acutely to various oxygen tension. The heavy lines pertain to man acclimatized near sea level, the light lines after acclimatization to 9500 ft.

TABLE 6. BREATH-HOLDING TIME AT ALTITUDE (PERCENT OF SEA LEVEL VALUE)

EXPOSED ACUTELY ¹							ACCLIMATIZED								
Authority	No. of subj.	Altitude X 10 ³ Ft.					Authority	No. of Subj.	Altitude X 10 ³ Ft.						
		0	5	10	15	18			0	6	7	9.5	14	16	21
Engel et al (31)	40	100	82	68	59	54	Schneider (20)	1	100	55			33		
Rodbard (32)	80	100	94	76	72	56	Hingston (34,	1	100		62		58	31	22
Otis et al (19)	8	100	81	66	59	54	35)								
Brown (33)	212	100				58	Our data	3	100			50			
Average (not weighted)		100	86	70	63	56									

¹ The values at these particular altitudes were in part obtained by interpolation of the data.

When breathing air, however, the breath-holding time is in addition affected by the hypoxic stimulus. Even so the decreasing buffering capacity during the process of acclimatization should exert itself by reducing the breath-holding time as was long ago observed by Schneider (20). Table 6 attempts to summarize the recent investigation of breath-holding time during acute exposure to low oxygen. These data are in

very good agreement and are plotted in figure 8. When this curve is compared to the relatively few breath-holding data known for acclimatized man (20, 34, 35) a definite reduction in breath-holding time is indicated. This decrease in breath-holding time with acclimatization may to some extent be explained by the reduced buffering capacity but may in part be counteracted by a reduced sensitivity to low oxygen.

DISCUSSION

Experimental evidence and data from the literature are compared and indicate that the alveolar air composition and consequently the ventilation response of the respiratory system differ between acclimatized and unacclimatized man for any altitude above sea level. In general it may be stated that the acclimatized subject has a greater ventilation and consequently a lower $p\text{CO}_2$ and a higher $p\text{O}_2$ compared to an unacclimatized individual exposed acutely for one hour to the same altitude.

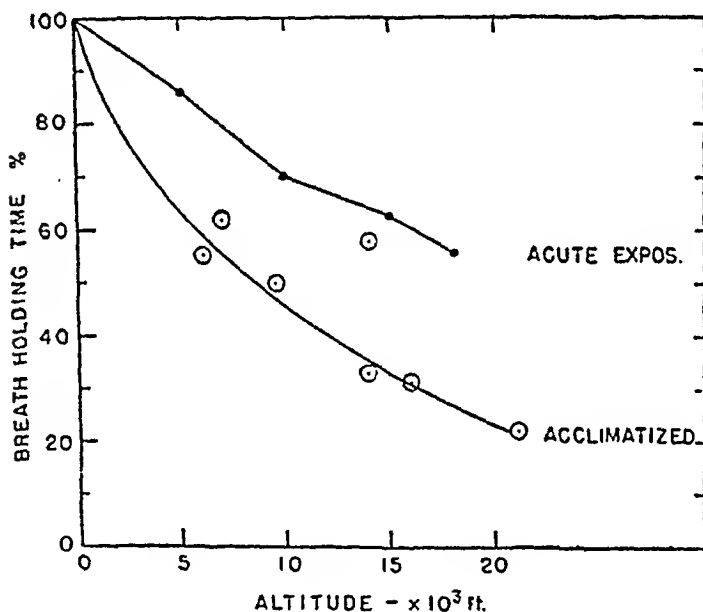


FIG. 8. BREATH HOLDING TIME as a function of altitude in man acutely exposed and acclimatized. Data taken from table 6.

If the rate of respiratory acclimatization is appraised by the alveolar CO_2 level, then our data indicate that this process requires 4 days at an altitude of 10,000 ft. while the data of Douglas *et al.* (18) indicate at least twice as long at 14,000 ft. This ventilatory inhibition, which is thus gradually overcome during the adaptation process, is most simply explained by the respiratory alkalosis incurred from the hypoxic hyperventilation of the acute exposure. With the excretion of base the $p\text{H}$ gradually returns to normal during the acclimatization process and the ventilatory inhibition is gradually diminished.

Direct and indirect (from alveolar air composition) measurements of the ventilation indicate no measurable change until man is acutely exposed to altitudes above 10,000 to 12,000 ft. At this altitude the alveolar and arterial $p\text{O}_2$ tensions are between 50 and 60 mm. Hg. This is the approximate threshold response described by Schmidt and Comroe (21) for the chemoreceptors in dogs. On the other hand, v. Euler, Liljestrand and Zotterman (22), recording the activity of the sinus nerve, place

the oxygen threshold much higher. This is supported by our curve of acclimatized man which indicates relative hyperventilation as soon as pO_2 drops below sea level values (fig. 2). Thus we must look for an inhibition which temporarily (approximately 1 hour) prevents hyperventilation when man is acutely exposed to altitude less than 10,000 to 12,000 ft. or to equivalent oxygen pressures at sea level. The only apparent change is the desaturation of the arterial blood which brings about a small increase in the arterial pH . One might argue that not until secondary compensations take place which return the pH to normal level would the hypoxic stimulus be able to exert its effect. That such compensatory changes can occur in a relatively short time has been shown recently by Bjurstedt (23) who measured the pH continuously in dogs exposed to low oxygen.

Thus the conflicting views as to the hypoxic threshold of the chemoreceptor may be reconciled by considering that this hypoxic drive upon the acute exposure up to approximately 10,000 to 12,000 ft. altitude (13-14% O_2 at sea level) can be detected by an increase in action potentials from the chemoreceptor (22) but is masked by the desaturation alkalosis if measured by increase in ventilation volume (21).

However, when alveolar oxygen tensions lower than 50-60 mm. Hg are encountered the chemoreceptor drive becomes enough to overcome the inhibitory desaturation alkalosis and now produces an increased ventilation. The recent findings of Bjurstedt (23) that the chemoreceptor drive is actually potentiated by the resulting arterial alkalosis make it hard to explain the unresponsiveness in man acutely exposed to inspired oxygen tension above 13 to 14 per cent oxygen.

The maintenance of the relative hyperventilation at altitude has generally been attributed to the increase activity of the chemoreceptors. When after an acute exposure to altitude the subject is again returned to normal oxygen concentration the ventilation is immediately reduced to normal and even below normal depending upon the alkalinity incurred under the previous hyperventilation. In fact this subnormal ventilation after a stay at 22,000 ft. may last as long as half an hour (6).

However, if acclimatization to altitude has occurred, then a sudden removal to sea-level oxygen values does not reduce the ventilation immediately. This was long ago observed by Douglas *et al.* (18) and others, and most recently by Houston and Riley (8). Hyperventilation is maintained at sea level and gradually diminishes over a period of several days. This might suggest that after acclimatization to altitude the chemoreceptor activity is reduced and thus removal of the hypoxic stimulus has little effect upon the ventilation. Bjurstedt (23) has recently come to this conclusion in studies on dogs where he was able to differentiate between the centrogenic and chemoreflex control of ventilation during various stages of acclimatization. His findings indicate that the chemoreflex drive is all important during the acute exposure to hypoxia and is actually potentiated by concomitant alkalosis. After secondary compensations set in and return the arterial pH to normal, the chemoreflex drive diminishes and the centrogenic drive is supernormal and is largely responsible for the maintained hyperventilation after acclimatization to altitude.

Thus, the respiratory system response, according to this concept, is very similar to that at sea level with the exception that due to the lowered buffer capacity ($BHCO_3$ content) it must, according to the Henderson-Hasselbalch equation, become more

sensitive to equal change in CO_2 as long as the $p\text{H}$ is considered as one of the stimulatory factors acting upon the centrogenic drive.

Consequently, breathing pure oxygen after acclimatization to altitude or suddenly returning to sea level might reduce part of the already insignificant chemoreflex drive, but any reduction in ventilation will have a profound effect upon the acid-base balance and the immediate fall in $p\text{H}$ and rise in $p\text{CO}_2$ will stimulate the respiratory center and thus prevent the drop in ventilation. Thus the same factor which operates to maintain a constant alveolar $p\text{CO}_2$ and arterial $p\text{H}$ at sea level also operates to maintain the same arterial $p\text{H}$ after acclimatization to altitude. The chemoreceptors play only a temporary part in the acclimatization process to high altitude in producing enough over-ventilation to lower the alkali reserve. Once this has been accomplished and the arterial $p\text{H}$ has returned to normal, the centrogenic drive is able to maintain this hyperventilation by its greater sensitivity to CO_2 , this in turn being merely a reflection of the lowered buffering capacity. On such a basis one may find a ready explanation for the altered response of the respiratory system to inhalation of CO_2 , breath-holding time and breaking point concentrations described above. Gray (36), on the other hand, leans toward the idea that prolonged acapnia as seen in altitude acclimatization increase the sensitivity to CO_2 per se, while the sensitivity of the respiratory system to $p\text{H}$ remains unaltered.

If the chemoreceptor drive is relatively unimportant once acclimatization has been achieved then one should be able to simulate the respiratory responses of an altitude-acclimatized individual by any means which would induce a lowering of the alkali reserves at sea level. This could be achieved by prolonged hyperventilation or ammonium chloride ingestion. The effects of prolonged hyperventilation (24 hours) in a Drinker respirator have recently been reported by Brown *et al.* (37). They show in 3 subjects a considerable reduction in the serum bicarbonate level accompanied by an increased sensitivity to inspired CO_2 similar to the response reported in our observations at high altitude. Furthermore, after leaving the respirator involuntary hyperventilation was maintained for a considerable period as seen also in people returning from prolonged stays at high altitudes. Preliminary experiments in this laboratory have shown that daily ingestion of 15 gm. of NH_4Cl for 3 days reduces not only the breath-holding time but also the alveolar $p\text{CO}_2$ at the breaking point of breath-holding in a manner similar to that observed after acclimatization to 10,000 ft. These experiments suggest, as has been pointed out by others, that a large part of the respiratory adaptations concerned with acclimatization to altitude is concerned with an adaptation to a lowered $p\text{CO}_2$ and a reduced buffer system of the blood which in turn raises the $p\text{O}_2$ tension and also prevents large fluctuations in O_2 tensions which are encountered during stresses such as work or voluntary apnea.

SUMMARY

With the aid of an O_2 - CO_2 diagram data are presented which allow one to predict the alveolar O_2 and CO_2 composition as well as the relative alveolar ventilation 1) when man is exposed acutely to any altitude, 2) during the process of respiratory acclimatization at any altitude and 3) when acclimatization is complete. In addition, the alveolar pathways can be predicted and described once man is acclima-

matized to any particular altitude and is then suddenly exposed to higher or lower oxygen pressures. These predictions were in part verified by an acclimatization study carried on at an altitude of 9500 ft. for a 3-week period.

Evidence is presented for a pO_2 threshold of the chemoreceptor drive of ventilation at approximately 100 mm. Hg. This is observed in the ventilation curve of people acclimatized to altitude. However, in *acute exposures* where the alveolar pO_2 is reduced to 50 to 60 mm. Hg this hyperventilation response is completely inhibited for at least one hour. This inhibition is explained as a result of the pH rise due to the decreased oxyhemoglobin saturation. If the alveolar pO_2 in acute exposure falls below 50 to 60 mm. Hg, immediate hyperventilation occurs. Exposures of more than one hour at 9500 ft. result in a lowering of the pCO_2 exponentially with time. Final levels are reached after 3 or 4 days.

After respiratory acclimatization to 9500 ft. the respiratory system becomes more sensitive to CO_2 . This response was tested by breathing various CO_2 mixtures and analyzing the alveolar air after breath holding. Data are presented which show the reduction of breath-holding time during acclimatization to various altitudes. The various findings emphasize that a large part of the respiratory acclimatization to high altitudes is an adaptation to a lowered CO_2 tension.

The authors express their gratitude to Dr. W. O. Fenn, who in various ways has contributed much to this work.

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INHIBITION OF BRAIN DEHYDROGENASES BY 'ANTICHOLINESTERASES'¹

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THE pharmacological actions of the anticholinesterase drugs, eserine sulphate (ES), diisopropylfluorophosphate (DFP) and tetraethylpyrophosphate (TEP), are usually attributed to their inhibition of cholinesterase (ChE). Some workers (1-3) have recognized that other actions might occur, and some (4-6) have found a lack of parallelism between pharmacologic effects and ChE inhibition. Other work in this laboratory (7, 8) has shown that these drugs inhibit the respiration of frog brain and nerve in concentrations which alter their electrical activity, as is also the case for methoxyfluoroacetate (MFA), which is not a ChE inhibitor. Yet the fluorophosphonates, tested on many purified enzyme systems *in vitro*, were found (9) inactive on all respiratory and other enzymes save only ChE and related esterases. It is, however, not exceptional for purified systems to be more resistant to damage than are less pure tissue extracts (e.g. 10), and even fluoroacetate was found (11) inert to most purified enzyme systems. We have accordingly tested these agents on brain and nerve brei, using the Thunberg methylene blue (MB) technique to reveal specific action on dehydrogenases. Marked inhibition of several of these has been found.

METHODS AND RESULTS

Cattle sciatic nerves (obtained fresh at Swift and Co., courtesy of Mr. Keefer and Dr. Brewer) were cleaned, frozen, and finally powdered in dry ice; dog sciatics were homogenized (Potter-Elvehjem homogenizer) fresh in iced saline. Brains of adult white rats were excised immediately after decapitation, a portion used for dry-weight determinations, and the remainder homogenized (in a mortar by hand) in Ringer's solution. This was always completed in less than a minute. The suspension, made up to 20 mg. fresh tissue per ml. of Ringer's solution, was dialyzed in a cellophane bag against 10 volumes of Ringer, to remove substrates, by shaking at 130 strokes per minute for 3 hours at room temperature. To 1 ml. of dialyzed tissue suspension were added 0.3 ml. of substrate (analytic grade), 0.2 ml. of inhibitor, 0.1 ml. of 1:5000 MB, and physiological saline to a final volume of 2 ml. The pH was always approximately 7.0. Each Thunberg tube was at once evacuated, placed in a water bath at 37°C., and decoloration time noted. The percentage of inhibition or other ratio was calculated as:

$$100 - 100 \times \frac{\text{minutes reduction time of substrate}}{\text{minutes reduction time of substrate plus inhibitor}}$$

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¹ Performed under contract between the Office of Naval Research and the University of Chicago.

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Early experiments were made on tissue kept frozen in dry ice, but this procedure was abandoned when it was found to injure the enzymes. This is shown in table 1. Except for succinate and α -ketoglutarate in infant brain, all reductions are seriously retarded by freezing. Frozen cattle brain seemed somewhat more active than frozen dog brain; both were about $\frac{1}{5}$ as active as fresh rat brain, and fresh nerve was only $\frac{1}{10}$ as active as fresh brain. Table 1 also shows clearly the different rates at which various substrates are acted upon by a given brain homogenate, and that these various activities are altered in relation to each other by age and by freezing. Glutamate and fumarate seem to inhibit. Salts are important, and if water is used instead of isotonic saline, as in these early experiments, the activity of some dehydrogenases (citrate, glucose, lactate) is cut to 10 to 15 per cent (cf. 11a). Infant brain is defi-

TABLE 1. INFLUENCE OF AGE; INJURY BY FREEZING
Non-dialyzed rat brain; in water

	INFANT (3-DAY)			YOUNG (22-DAY)		ADULT		
	Fresh		Frozen (1 day)	Fresh		Fresh		Frozen (5 days)
	13	12	12	16	18	23	23	23
Dry wt., %.....	23	23	23	23	23	20	9	9
Mg. wet wt./ml. susp.....	2.9	2.7	2.7	3.7	4.1	4.7	2.1	2.1
Mg. dry wt./ml. susp.....								
<i>Addition (M/67)</i>								
Blank.....	53 ¹	130	180	20	45	36	180	180
Oxaloacetate.....	27	22	50	11	11	5	47	180
Succinate.....	17	21	20	7	8	6	10	60
Glutamate.....	180	180	180	180	180		180	180
Malate.....	32	42	68	16	11	23	52	180
Citrate.....	50	60	78	23	21	19	120	180
Lactate.....	26	34	77	8	14	14	140	180
Glucose.....	29	40		18	9	17	55	180
Pyruvate.....	24	39	78	17	18	13	58	180
α -Ketoglutarate.....	45	64	60	25	25	21	120	180
Fumarate.....	60	180		180	9		180	180
Maleate.....	80	100	180	26	22	69	120	180

¹ Reduction time in minutes. 180 = no reduction in 180 minutes.

nitely less active than adult on a wet weight basis, but is comparable in terms of dry weight (12).

Table 2 summarizes activities for particular substrates and rat brain under standard conditions. The independent behavior of each substrate strongly suggests that reduction rates are being determined by the activities of specific dehydrogenases rather than by some common factor, such as coenzyme (12a). That sufficient coenzyme was present so that it was not a limiting factor was further proven by adding coenzyme I (kindly supplied by Dr. F. Schlenk), 20 γ per tube, to dialyzed suspensions. This did not hasten reduction time for substrates nor alter the inhibition produced by any inhibitor.

The critical experiments with inhibitors are summarized in table 3. Controls with hydrolysis products or associated ions of the inhibitors were negative. Sodium

fluoroacetate (NaFA) was practically inactive on brain or nerve, even when tested on homogenates in water with cells disrupted. It is striking that, for most substrates and at most inhibitor concentrations, the 'metabolic' inhibitor, MFA, is less effective against dehydrogenases than are the 'anticholinesterases', DFP and TEP. DFP is, on the whole but not uniformly (e.g. malate), somewhat more effective than TEP; ES is distinctly less so. The abilities of these drugs to inhibit tissue respiration *in*

TABLE 2. RELATIVE ACTIVITIES OF SUBSTRATES WITH DIALYZED ADULT RAT BRAIN SUSPENSIONS

SUBSTRATE ¹	NO. OF TESTS	AV. REDUCTION TIME	RELATIVE ACTIVITY
		min.	%
Oxaloacetate.....	6	3.4	100
Succinate.....	17	4.5	76
Malate.....	6	5.7	60
Lactate.....	14	6.1	56
Citrate.....	6	6.2	55
Glutamate.....	5	7.2	47
Glucose.....	6	7.2	47
α -Ketoglutarate.....	2	11.0	31
Maleate.....	2	15.5	28
Pyruvate.....	2	15.5	28
Fumarate.....	2	17.5	19
Blank.....	32	18.1	

¹ All substrates M/67. 20 mg. brain/ml.

TABLE 3

SUBSTRATE ¹ (M/67)	PERCENTAGE INHIBITION OF MB REDUCTION																
	M/45	M/200			M/450	M/800			M/1000			M/1500			M/10,000		
	ES	DFP	TEP	MFA	ES	DFP	TEP	MFA	DFP	TEP	MFA	DFP	TEP	MFA	DFP	TEP	MFA
Oxaloacetate.....		40	40	25	0				25	14	14	25	14	14	0	0	0
Succinate.....	71	67	52	20	0	30	0	11	22	0	11	22	0	11	12	0	0
Malate.....	73	76	73	40	0	50	59	37	31	52	28	26	33	31	0	0	21
Lactate.....	86	100	100	18	0	70	47	15	56	45	0	41	15	0	17	0	0
Citrate.....	50	60	39	14	0	10	39	14	5	26	14	0	7	14	0	0	14
Glutamate.....	9	28	50	17	0		9	9	17	0	9	17	0	0	0	0	0
Glucose.....	100	100	100	46	0	98	71	22	88	60	22	76	45	12	12	25	12

¹ The substrates fumarate and maleate did not hasten MB reduction of undialyzed brain; pyruvate and α -ketoglutarate were slightly active.

vitro, to inhibit ChE, or to produce symptoms in an animal are not in constant ratio to each other from case to case, even for the same phenomenon, so that extensive comparisons here would not be profitable.

There is little correlation between the activity of a dehydrogenase system and the ease with which it is inhibited by all these drugs, and, even at the highest drug concentrations used, the percentage inhibition for various substrates may range from 100 to under 30. Glucose is consistently the most fully inhibited, glutamate almost as regularly the least. Succinate is relatively more sensitive to DFP (and to ES)

than to TEP or MFA, especially at the lower range of inhibitor concentrations. Other particular instances of high sensitivity (malate to weak MFA) or low sensitivity (citrate to DFP) are manifest in the table. Dehydrogenases in the infant brain seem to be somewhat more susceptible than in the adult. In 2 experiments at $10^{-4}M$ concentration, MFA gave about one-fifth inhibition with lactate and TEP about one-third. Dog nerve also showed significant inhibition of succinate and lactate oxidation by TEP, even at $10^{-4}M$. At $0.005M$, DFP and MFA inhibited succinate by 40 per cent, TEP by 60 per cent, and chloretone not at all.

In a few experiments, ribonucleic or desoxyribonucleic acid (200 γ /tube) inhibited lactate dehydrogenase of brain by a third or more. (Compare liver, 13a.) A nucleic acid combined with one of the above drugs ($0.001M$) roughly doubled the drug inhibition.

Of the systems tested, the dehydrogenase for succinate does not require coenzyme (or flavoprotein) for activity; all the others do. The latter, except for glu-

TABLE 4. INHIBITION OF MIXED SUBSTRATES

SUBSTRATE	REDUCTION TIME ¹ (MIN.)		% INHIBITION
	No addition	TEP $0.001M$	
Lactate.....	6.7	9.5	30
Succinate.....	5.5	5.5	0
Both.....	3.7	4.0	10
Both, calculated.....	3.1	3.6	15
Glucose.....	7.2	15.0	50
Succinate.....	4.2	4.2	0
Both.....	2.8	3.6	20
Both, calculated.....	2.6	3.2	20

¹ Values are adjusted for 4 to 6 comparable experiments using substrates at $M/67$, $M/144$, or mixed concentrations.

tamate, tended to be more easily inhibited. That the drug action is not on coenzyme is shown, however, not only by the lack of inhibition of the glutamate system, but also by the earlier-mentioned inability of added coenzyme to alter the picture. Further evidence that the inhibitors act on the apoenzyme is supplied by their behavior with mixed substrates.

When either lactate or glucose, both of which are inhibited by $0.001M$ TEP, is mixed with succinate, which is not inhibited, reduction time is shorter than that for either alone, but rather greater than that calculated for their complete summation ($\frac{1}{t_A} + \frac{1}{t_B} = \frac{1}{t_{A+B}}$) (table 4). This indicates that the two reducing systems are largely, but not completely, independent. If A is partially inhibited by a particular drug and B is not, reduction time for $A + B$ should approach that for A by a calculable amount when no interdependence exists. Actually, as table 4 shows, for lactate or glucose as A and succinate as B , this is roughly the case. Results are alike whether the inhibited or the uninhibited substrate is added first. It thus appears that TEP can act on one dehydrogenase, leaving another unaffected.

DISCUSSION

Apart from the poisoning of heavy metal proteins with cyanide or azide, two modes of action of inhibitors on respiratory enzymes have been described. Chlorthalidone and barbiturates leave the dehydrogenases largely unimpaired, and probably affect a flavoprotein acting on cytochrome (13, 14). Morphine, codeine and thebaine, on the other hand, inhibit certain dehydrogenases of brain (15); this action has been suggested for diethylstilbestrol (16). The drugs here tested act also on the dehydrogenases. Whether several steps are involved in the substrate oxidation, as may well occur with glucose or oxaloacetate, or only one, as for lactate, is not important for the present findings. What is essential is that drugs regularly identified as anticholinesterases, and studied almost solely in terms of this property, are here shown to be powerful dehydrogenase inhibitors as well. Their ability to alter physiological behavior of nerve and brain follows their action on respiration more closely than their action on ChE (7, 8). It seems unjustifiable to attribute pharmacological effects specifically to changes in the acetylcholine system.

SUMMARY

The dehydrogenase activity of brain and nerve was studied with the methylene blue technique. Relative activities with 11 substrates are presented. Age had little influence on brain dehydrogenases; freezing decreased activity to about one-fifth. Nerve was about one-tenth as active as brain. The 'anticholinesterase' drugs, DFP, TEP, and ES, are inhibitors of brain and nerve dehydrogenases, the two former being in general even more powerful than the 'metabolic inhibitor' MFA. Dehydrogenase inhibition is specific in pattern for each drug; even at 10^{-4} M drug concentration the oxidation of particular substrates may be inhibited by 10 to 25 per cent.

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DESCENDING RESPIRATORY PATHWAYS IN THE CERVICAL SPINAL CORD

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IT IS generally accepted that under normal conditions the activity of each half of the diaphragm is a consequence of excitation by the ipsilateral phrenic motoneurons, which in turn are activated by impulses originating in the medullary respiratory center and passing via descending fibers which are uncrossed (*viz.*, make direct connection with their ipsilateral phrenic nucleus).

The existence of some crossed connections in this system was first demonstrated physiologically more than fifty years ago by Langendorff (1) and Schiff (2). They showed that under certain experimental conditions the respiratory discharge descending on one side of the cervical spinal cord can excite the contralateral phrenic motoneurons. Porter (3) further demonstrated that this crossing occurred at the level of the phrenic nuclei. In 1912, Deason and Robb (4), working under the direction of A. J. Carlson, reported that the phenomenon of crossed excitation is a function of the intensity of discharge from the bulbar center. Still more recently, Rosenblueth and Ortiz (5) repeated Porter's work, with some modification of his methods, and concluded that the crossed phenomenon reveals properties of neurone paths which differ in quality from the conduction of nerve impulses and, in direct contrast to Deason and Robb, found that with maneuvers which markedly augment the intensity of the respiratory effort (e.g. asphyxia) they failed to demonstrate any crossed activity under the conditions of their experiments. Pitts (6) suggests that some hitherto unknown properties of the central nervous system underly the crossed phrenic phenomenon of Porter and that its elucidation would be a valuable contribution.

After reviewing the literature which bears on the points under discussion, it was found that there is disagreement as to the functional importance of the crossed connections. In particular there is disagreement as to the conditions necessary for the effective excitation of phrenic motoneurons by impulses which traverse crossed pathways. The data presented here have some bearing upon these points. We have studied the activity of descending respiratory pathways as reflected by the activity in each hemidiaphragm. The method of recording diaphragmatic activity is the principal point of difference between our procedure and those of previous investigators. Rather than observing the movements of the diaphragm or making mechanical records of its contractions, we have utilized the action currents of the two halves of the diaphragmatic muscle, amplified differentially and visualized by means of the cathode ray oscilloscope. Employing this method we have studied the effects on diaphragmatic activity both of enhancing and of diminishing the respiratory effort in cats and rabbits immediately following partial transection of the cervical spinal cord above the level of the phrenic motor nuclei.

METHODS

Sixteen acute experiments were performed, 8 with cats and 8 with rabbits. Some of the preparations had been decerebrated under ether anesthesia by the method of Schmidt (7). Others had been anesthetized with sodium pentobarbital ('Nembutal' Abbott), or diallylbarbituric acid with urethane and monoethylurea, its route of administration, and its dose are given in the abbreviated protocols presented below (see also figs. 3 and 4).

The vagal nerves remained intact, except in one rabbit in which they were transected near the end of the experiment (*exper. 1*). After inserting a glass tracheal cannula, each preparation was placed in the prone position with neck flexed, a partial laminectomy of the axis performed, and a partial transverse section of the cord made at the lower half of the second cervical segment. The preparation was then placed in the supine position and a midline abdominal incision made caudad from the xiphoid cartilage. The xiphoid cartilage was raised and clamped, and the abdominal wall and liver were carefully retracted. This maneuver sufficiently exposed the abdominal surface of the diaphragm to permit the symmetrical placement in it of two pairs of small electrodes, one pair lateral to the central tendon on each side. The electrodes were short loops prepared from fine insect pins. Each pair was connected, by means of the fine (no. 40) insulated wire, with the input terminals of a condenser-coupled differential amplifier of short time constant. The amplified diaphragmatic action potentials from each hemidiaphragm were visualized by means of cathode ray tubes and photographed on running film (see figs).

Each experiment consisted of recording and comparing the action potentials of each hemidiaphragm *a*) in the control state and *b*) during various procedures which alter the respiratory effort: artificial hyperventilation and lung inflation to depress it; and tracheal occlusion, rebreathing, and lung deflation to augment it.

Toward the end of each experiment one or both of two tests that will be described below were made to determine quantitatively the degree of independence of the simultaneous recordings from the two sides of the diaphragm. The upper cervical cord was then removed and placed in 4 per cent formaldehyde. The extent of the lesion at C₂ was determined subsequently by analysis of serial sections stained by the Lillie variant of the Weigert method (8). The lesions for each experiment are shown in figures 3 and 4, in which the minimal areas of indubitable transection appear in black. A few distorted fibers in an edematous and/or hemorrhagic matrix remained in the stippled areas, and it is plausible to believe that the injury to them was sufficient to block the conduction of impulses. It is, of course, possible that functional block extended somewhat beyond the limits of the black and stippled areas.

RESULTS

The findings from a typical experiment on a cat (*exper. 1*) anesthetized with sodium pentobarbital (30 mg/kg.) are illustrated in figure 1. As in all the figures, action currents from the right hemidiaphragm are shown in the upper tracing, those from the left hemidiaphragm, in the lower tracing. The time-line gives 1-second intervals and the voltage scale is as indicated. The tracing on the upper left shows the extent of the transverse lesion at C₂, as revealed by the histological analysis of serial sections.

As in all the figures, the orientation is cord dorsum to the right, the right side of the cord above and the left below to correspond with the action current records. It will be seen that in this particular experiment the lesion involved almost the entire anterior and lateral columns on the left, and a portion of the right anterior column as well.

The diaphragmatic action potentials of two inspiratory efforts are shown in the control record (*a*). In addition, the incidental recording of the QRS complex of the electrocardiogram is visible. The noteworthy feature of this record is the significant diaphragmatic activity on the side of the cord lesion. The activity on the side of the lesion, and on the opposite side as well, could readily be augmented; record *b* shows that significant enhancement above the control level was induced by rebreathing for 8 seconds; and record *d* illustrates the prompt augmentation produced by lung defla-

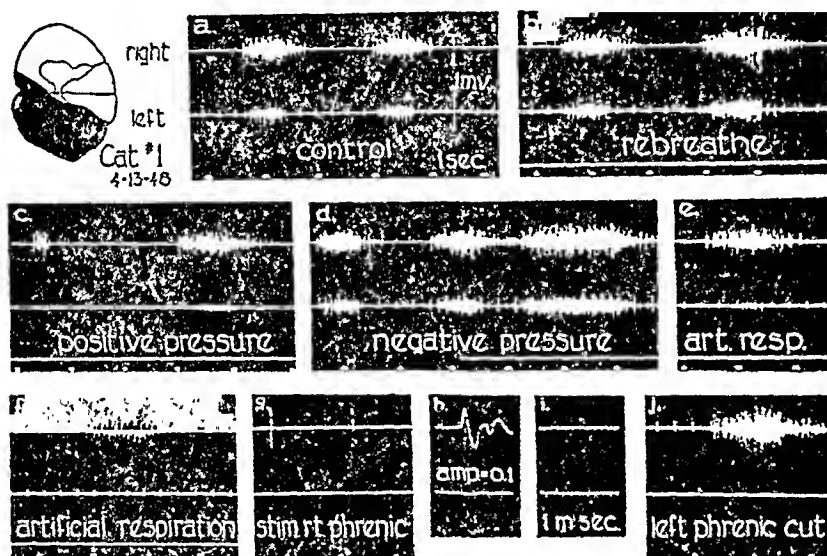


Fig. 1. ILLUSTRATIVE DATA from a typical experiment on a cat (pentobarbital anesthesia) Upper left: tracing of reconstruction of the partial transverse spinal cord section at C₂, oriented with cord dorsum to the right and right side at top. Oscillograms: action potentials from the 2 sides of the diaphragm, right above and left below to correspond with the drawing of the spinal cord. Time and voltage scales as indicated. Record *i* gives time-line for record *h*. See text for further details.

tion (application of negative pressure to the trachea). Likewise, the activity on both sides could readily be decreased. It is of particular interest that it proved easy differentially to abolish activity on the side of the lesion, that is, to abolish action currents on the side of the lesion while maintaining significant activity on the opposite side. One example may be seen in record *c*, which shows the first two inspiratory efforts that followed an apnea produced by lung inflation (positive pressure applied to trachea). The first inspiratory effort was almost entirely confined to the side opposite the spinal lesion; in the second, the activity on the side of the lesion was differentially reduced in comparison with the control state (record *a*). A second example is shown in records *e* and *f*. Hyperventilation artificially administered first produced differential reduction of activity on the side of injury (*e*), and then differential abolition of that activity (*f*). Continued hyperventilation eventually abolished activity

on the intact side. Cessation of hyperventilation was followed by similar changes in reversed sequence.

An essential control was determination of the independence of the simultaneous action current recordings from the two sides of the diaphragm. Accordingly, one or both of two tests were made in each experiment. Both tests were performed in the experiment now being described.

The first is illustrated by records *g* and *h*. Like records *a-f* and *j*, record *g* was taken on running film. Record *h* represents a single, relatively fast sweep, for which the time-line is shown in *i*. Both *g* and *h* were taken at one-tenth the standard amplification for the experiment as shown in *a*. The deflections show the action currents

TABLE 1. SUMMARIZATION OF DATA

Cats

Essential data from each of the experiments are summarized in tables 1 and 2 and in figures 3 and 4.

NO.	DATE	EXPER. PREPARATION AND CONDITION	EXTENT OF LESION GREATER OR LESSER THAN HEMI-SECTION (FIG. 3)	CROSSED ACTIVITY PRESENT IMMEDIATELY AFTER LESION	CROSSED-ACTIVITY CAPABLE OF BEING:		PER-CENTAGE INTER-ACTION BETWEEN DIFFERENTIAL AMPLIFIERS
					ENHANCED OR BROUGHT IN	ABOLISHED	
1	4-13-48	Sodium pentobarbital; good condition	=	Yes	Yes	Yes	ca. 2.5
2	4-23-48 A	Sodium pentobarbital; good condition	<	Yes	Yes	Yes	< 1.7
3	4-23-48 B	Sodium pentobarbital; poor condition	<	Yes			< 2
4	4-24-48	Sodium pentobarbital; good condition	>	Yes	Yes	Yes	< 3
5	4-27-48	Sodium pentobarbital; good condition	>	Yes	Yes	Yes	1
6	5-3-48	Sodium pentobarbital; good condition	>	Yes	Yes	Yes	3
7	4-30-48	Decerebrated, good condition	>	Yes	Yes	Yes	< 2
8	5-11-48	Decerebrated; good condition	>	Yes, but small	Yes	Yes	< 0.3

produced by application of single shocks to the phrenic nerve in the neck on the intact (right) side. In both *g* and *h*, the large action current produced in the right hemidiaphragm was reflected by only a small deflection in the recording device connected with the left hemidiaphragm. The second test is section of the phrenic on the side of the cord lesion. As shown in record *j*, a large inspiratory effect on the intact side then produced little or no deflection in the record from the side corresponding to the cord lesion. In this experiment the interaction was conservatively estimated to be 2.5 per cent. It was considerably smaller in many of the other experiments.

It may be noted that the small or negligible amount of interaction had three implications: 1) physical spread of action current from muscle fibers in one hemidiaphragm to the recording electrodes in the opposite hemidiaphragm was small or

negligible; 2) physical interaction between the two channels of amplification was small or negligible; and 3) there was no evidence that fibers of the phrenic nerve cross peripherally (cf. Rosenblueth and Ortiz).

Results from an experiment on a rabbit (*exper. 6*) anesthetized with urethane are presented in figure 2. The diagram shows that the spinal lesion on the right side at C_2 was somewhat less than a hemisection. Attention may be directed first to the controls in which the degree of independence of the recordings from the two sides of the diaphragm was tested (records *f-j*). Record *f* shows that very large action potentials in the left hemidiaphragm, produced by stimulation of the left phrenic, produced scarcely visible deflections in the recording from the right. (The downward deflection produced by each volley in the left record was so large and rapid that it did not photograph sufficiently well to be reproduced.)

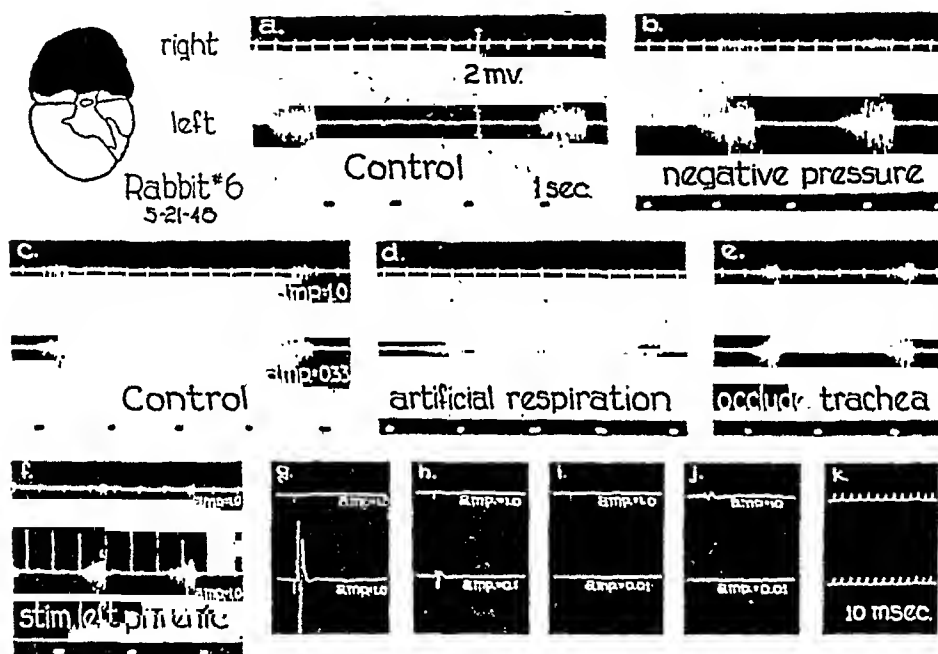


Fig. 2. ILLUSTRATIVE DATA from a typical experiment on a rabbit (urethane anesthesia). Arrangement as in fig. 1. Record *k* gives time-line for records *g-j*. See text for further details.

The degree of interaction may be determined more quantitatively from records *g-j*. At the standard amplification of the experiment (record *g*), stimulation of the phrenic on the intact (left) side evoked in that side of the diaphragm an action potential so large that it was only partially recorded. Nevertheless, only a minimal deflection followed the shock artefact in the record from the opposite (right) hemidiaphragm. In the next two records the amplification on the intact side was reduced, first 10-fold (*h*), then 100-fold (*i*), while that on the side of the cord lesion remained unchanged. It may be seen that the response on the intact side to stimulation of its phrenic, when reduced 100-fold, was still about three times as great as the deflection which appeared in the records from the opposite side as shown in the upper tracings of records *g*, *h*, and *i*. When the amplification on the intact side was reduced 100-fold and that on the opposite side simultaneously increased 10-fold, as shown in record *j*, the response on the intact side was approximately three times the greater.

Thus the controls agree in showing that the interaction in this experiment was only about 1 in 300, or 0.3 per cent.

As shown in record *a*, at the beginning of this experiment diaphragmatic activity in the control state was confined to the hemidiaphragm contralateral to the spinal lesion. However, lung deflation produced by application of negative intratracheal pressure, as shown in record *b*, promptly induced activity on the side of the lesion. As a sequel to very prolonged lung deflation with accompanying rebreathing into a small dead space, the preparation went into a circulatory and respiratory crisis with apnea. It quickly recovered during a short period of artificial respiration. Subsequently, and for the duration of the experiment (30 min.), diaphragmatic activity was present on the side of the lesion in the control state (record *c*). This crossed

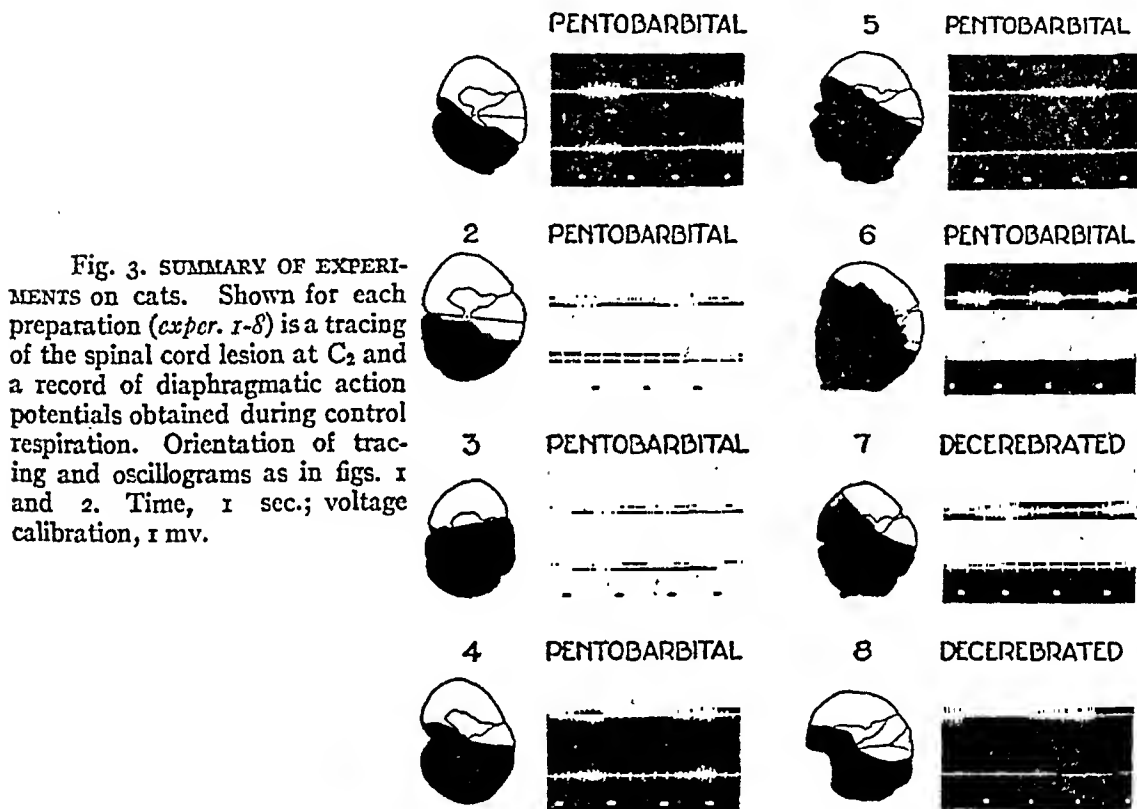


Fig. 3. SUMMARY OF EXPERIMENTS on cats. Shown for each preparation (*exper. 1-8*) is a tracing of the spinal cord lesion at C_2 and a record of diaphragmatic action potentials obtained during control respiration. Orientation of tracing and oscillograms as in figs. 1 and 2. Time, 1 sec.; voltage calibration, 1 mv.

activity could readily be differentially abolished during artificial hyperventilation (record *d*), and also during the first inspiratory efforts that broke through an apnea produced by lung inflation. It could be augmented significantly above the control level during the dyspnea produced by tracheal occlusion (record *e*), and also by forced deflation of the lungs.

DISCUSSION

Cats. In every experiment a significant amount of activity in the hemidiaphragm on the side of the spinal injury occurred during control respiration. The spinal lesions at C_2 varied from slightly less than a hemisection (*exper. 2*) to such an extensive injury as to leave intact only a part of the opposite lateral column (*exper. 6* and *7*).

In every experiment the crossed activity could be differentially abolished (i.e.

abolished with persistence of activity on the intact side) by artificial hyperventilation, or by forcible inflation of the lungs, or by both. These procedures, whatever else they may do, decrease the respiratory effort. In every experiment the crossed activity could be considerably augmented by rebreathing, forcible deflation of the lungs, and/or tracheal occlusion. These procedures, whatever else they may do, enhance the respiratory effort.

Rabbits. The rabbits differed quantitatively from the cats in that diaphragmatic activity on the side of the spinal lesion was initially present during control respiration in only 2 of the 8 preparations. Crossed respiration was present in 4 of 8 if one

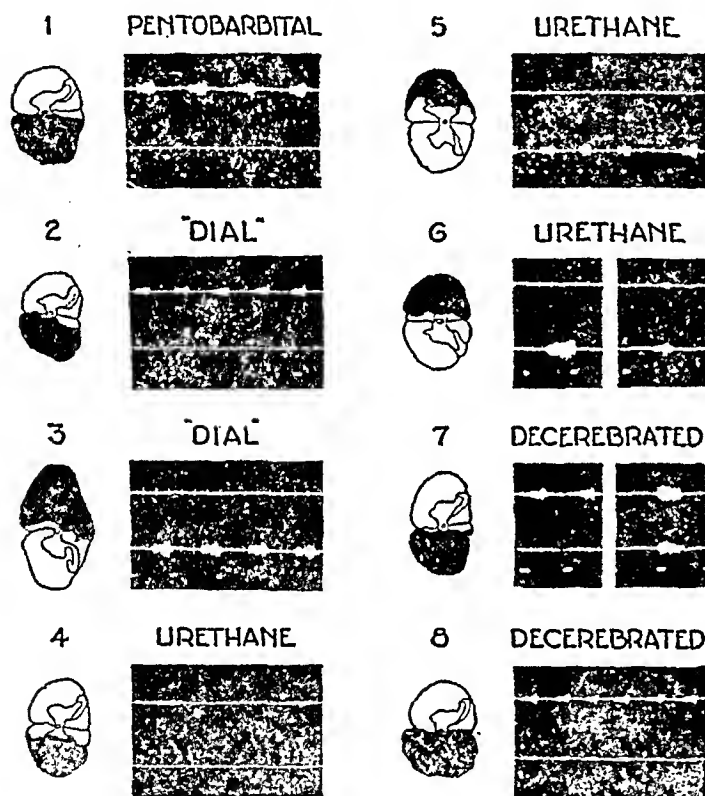


Fig. 4. SUMMARY OF EXPERIMENTS on rabbits. Arrangement same as fig. 3 except that voltage calibration indicates 2 mv.

includes *experiments 4 and 6*, in which crossing was initially absent but appeared during the course of the experiments.

In every case in which crossed respiration was absent during control periods, it could be induced in significant degree by the above-mentioned procedures which enhance the respiratory effort. In every case except one¹ in which crossed respiration was present in the control state, it could be differentially abolished by procedures which decrease the respiratory effort.

Bulbo-spinal fibers of respiratory function descending on each side of the cervical spinal cord make direct or indirect connections with neurons of both phrenic motor nuclei. From the standpoint of excitatory function, the connections with the crossed

¹ *Experiment 8* comprises a single exception to this statement. The respiration of the decerebrated preparation was irritative, presumably because of the presence of a blood clot found about the medulla at autopsy, and was very insensitive to reflex and chemical influences.

phrenic motoneurons are similar to but quantitatively somewhat less powerful than the connections with the ipsilateral motoneurons. Intensity of discharge from bulbar centers is a very important factor determining whether or not impulses in the crossing pathways effectively excite the crossed phrenic motoneurons. Effective excitation of phrenic motoneurons by impulses in the crossing pathways regularly occurs or can easily be induced, even when both the opposite phrenic nerve and the vagi are intact.

TABLE 2. SUMMARIZATION OF DATA

Rabbits

NO.	DATE	EXPERIMENTAL PREPARATION AND CONDITION	EXTENT OF LESION- GREATER OR LESSER THAN HEMI- SECTION (FIG. 4)	CROSSED- ACTIVITY PRESENT IMMEDIATELY AFTER LESION	CROSSED-ACTIVITY CAPABLE OF BEING:		PER- CENTAGE INTER- ACTION BETWEEN DIFFER- ENTIAL AMPLIFIERS
					Enhanced or brought in	Abol- ished	
1	4-15-48	Sodium pentobarbital; good condition	<	No	Yes		3
2	4-20-48	'Dial'; good condition	=	No	Yes		5±
3	5-28-48	'Dial'; good condition	=	No	Yes		0.3
4	5-19-48	Urethane; good con- dition	≤	No, but appeared spontane- ously soon after	Yes	Yes	1
5	5-20-48	Urethane; good con- dition	<	No	Yes		0.3
6	5-21-48	Urethane; poor con- dition at first, then improved	=	No	Yes		0.3
7	5-14-48	Decerebrated; condi- tion relatively poor due to clot about medulla	=	Yes	Yes	Yes	<11
8	5-18-48	Decerebrated; condi- tion same as in no. 7	=	Yes	Yes	No	0.1

SUMMARY

In acute experiments on decerebrated or anesthetized cats and rabbits, a spinal lesion approaching or exceeding a hemisection was made at the second cervical level. The discharge of the phrenic motoneurons on each side of the cord was then measured by simultaneous action potential records from each hemidiaphragm. Controls established the absence of significant interaction between the 2 recordings.

In each of 8 cats significant crossed (i.e. ipsilateral to lesion) diaphragmatic activity occurred in the control state, even when the spinal lesion far exceeded a hemisection. This crossed activity could be completely and differentially abolished by procedures which decrease respiratory effort (e.g. hyperventilation), and augmented

by procedures which enhance respiratory effort (e.g. rebreathing, negative intra-tracheal pressure). Similar results obtained for those rabbits (4 of 8) which exhibited crossed respiration in the control state. Procedures which enhance respiratory effort elicited significant crossed activity in the remaining rabbits.

In conclusion, bulbo-spinal fibers of respiratory function descend on the intact side of the cord and make connections with phrenic motoneurons of the opposite side. These connections are quantitatively less powerful than the uncrossed ones. Intensity of discharge from the respiratory center is an important factor determining whether impulses in the crossing pathways alone will effectively excite the phrenic motoneurons.

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